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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/19, C07K 14/52, 16/24, C12N 15/11 // 15/62, A61K 38/19

A2

(11) International Publication Number: WO 98/11226

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/US97/15315

(22) International Filing Date: 9 September 1997 (09.09.97)

(30) Priority Data:
60/025,724 10 September 1996 (10.09.96) US

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(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, ID, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MAMMALIAN CHEMOKINES, RELATED REAGENTS

(57) Abstract

Novel CC and CXC chemokines from humans, reagents related thereto including purified proteins, specific antibodies and nucleic acids encoding these chemokines are provided. Also provided are methods of making and using said reagents and diagnostic kits.

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MAMMALIAN CHEMOKINES; RELATED REAGENTS

FIELD OF THE INVENTION

The present invention contemplates compositions related to proteins which function in controlling development, differentiation, trafficking, and physiology of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides proteins which regulate or evidence development, differentiation, and function of various cell types, including hematopoietic cells.

BACKGROUND OF THE INVENTION

The circulating component of the mammalian circulatory system comprises various cell types, including red and white blood cells of the erythroid and myeloid cell lineages. See, e.g., Rapaport (1987) Introduction to Hematology (2d ed.) Lippincott, Philadelphia, PA; Jandl (1987) Blood: Textbook of Hematology, Little, Brown and Co., Boston, MA.; and Paul (ed.) (1993) Fundamental Immunology (3d ed.) Raven Press, N.Y.

For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network." Recent research has provided new insights into the inner workings of this network. While it remains clear that much of the response does, in fact, revolve around the network-like interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins, known as lymphokines, cytokines, or monokines, play a critical role in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which should lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system and other disorders.

Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the

proliferation, growth, and differentiation of the pluripotential hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages making up a complex immune system. These interactions between the cellular components are necessary for a healthy immune response. These different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

The chemokines are a large and diverse superfamily of proteins. The superfamily is subdivided into four branches, based upon whether the first two cysteines in the classical chemokine motif are adjacent (termed the "C-C" branch); spaced by an intervening residue ("C-X-C"); a new branch which lacks two cysteines in the corresponding motif, represented by the chemokines known as lymphotactins; and an even newer branch where there are three intervening residues, e.g., CX3C chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.

Many factors have been identified which influence the differentiation process of precursor cells, or regulate the physiology or migration properties of specific cell types. These observations indicate that other factors exist whose functions in immune function were heretofore unrecognized. These factors provide for biological activities whose spectra of effects may be distinct from known differentiation or activation factors. The absence of knowledge about the structural, biological, and physiological properties of the regulatory factors which regulate cell physiology in vivo prevents the modulation of the effects of such factors. Thus, medical conditions where regulation of the development or physiology of relevant cells is required remains unmanageable.

SUMMARY OF THE INVENTION

The present invention reveals the existence of a previously unknown class of chemokine-motif containing molecules which are hereby designated 61164 or 331D5 chemokine. Based on sequence analysis of the two chemokine protein sequences described below, it is apparent that 61164 belongs to the C-X-C chemokine family and 331D5 belongs to the CC chemokine family.

The present invention provides a composition of matter selected from the group consisting of: a substantially pure or recombinant protein or peptide which comprises a plurality of epitopes found on, and exhibits at least 90% sequence identity over a length of at least 12 contiguous amino acids to, a protein of mature SEQ ID NO: 6 or 8; a substantially pure polypeptide of natural mature SEQ ID NO: 6 or 8; a substantially pure or recombinant protein or peptide which comprises a plurality of epitopes found on, and exhibits at least 90% sequence identity over a length of at least 12 contiguous amino acids to, a protein of mature SEQ ID NO: 12 or 14; and a substantially pure polypeptide of natural mature SEQ ID NO: 12 or 14. In preferred embodiments the protein or polypeptide comprises a segment exhibiting sequence identity to a corresponding portion of SEQ ID NO: 6, 8, 12, or 14, and: the identity is at least about 90% identity and the portion is at least about 17 amino acids; the identity is at least about 80% identity and the portion is at least about 23 amino acids; or the identity is at least about 70% identity and the portion is at least about 30 amino acids. Alternatively, the composition of matter will be one where: the recombinant protein comprises a mature sequence of SEQ ID NO: 6, 8, 12 or 14; protein binds with specificity to an antibody generated against an immunogen selected from SEQ ID NO: 6, 8, 12, or 14; or the protein or peptide: is from a warm blooded animal selected from a mammal, including a primate; comprises a plurality of polypeptide segments of 8 amino acids of SEQ ID NO: 6, 8, 12, or 14; exhibits a plurality of portions exhibiting the identity; is a natural allelic variant of SEQ ID NO: 6 or 8; has a length at least

about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for primate protein of SEQ ID NO: 6; exhibits a sequence identity at least about 90% over a length of at least about 20 amino acids to SEQ ID NO: 6 or 8; exhibits at least two non-overlapping epitopes which are specific for a primate protein of SEQ ID NO: 12; exhibits a sequence identity at least about 90% over a length of at least about 20 amino acids to a SEQ ID NO: 12 or 14; is not glycosylated; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from a natural sequence; or is a deletion or insertion variant from a natural sequence. In particular embodiments, the composition comprises: a sterile protein or peptide as described; or the protein or peptide and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

The invention further provides such peptides in a fusion protein, e.g., comprising: mature protein sequence of SEQ ID NO: 6, 8, 12, or 14; a detection or purification tag, including a FLAG, His6, or Ig sequence; or sequence of another chemokine protein. Other aspects include a kit comprising a protein or polypeptide, and: a compartment comprising the protein or polypeptide; and/or instructions for use or disposal of reagents in the kit.

Other aspects of the invention are a binding compound comprising an antigen binding portion from an antibody and which binds with specificity to such natural proteins, wherein: the protein is a primate protein; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a peptide sequence of a mature polypeptide comprising sequence of SEQ ID NO: 6 or 8; is raised against a peptide sequence of a mature polypeptide comprising sequence of SEQ ID NO: 12 or 14; is raised to a purified peptide of at least 15 contiguous amino acids of SEQ ID NO: 6, 8, 12, or 14; is immunoselected; is a polyclonal antibody; binds to a denatured protein of SEQ ID NO: 6, 8, 12, or 14; exhibits a Kd to antigen of at least 30 μM; is

attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label. Kits are provided comprising the binding compound, and: a compartment comprising the binding compound; and/or instructions for use or disposal of reagents in the kit. In certain embodiments, the kit is capable of making a qualitative or quantitative analysis.

Another aspect of the invention is a composition comprising: a sterile binding compound as described, or the binding compound and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

Nucleic acid embodiments include an isolated or recombinant nucleic acid encoding a protein or peptide described above, wherein: the protein is from a mammal, including a primate; or the nucleic acid: encodes an antigenic peptide exhibiting a plurality of epitopes from any of SEQ ID NO: 6, 8, 12, or 14; comprises at least 70% identity to the mature protein coding portion of SEQ ID NO: 5, 7, 11, or 13; exhibits at least about 80% identity to a natural cDNA encoding the segment; is an expression vector; further comprises an origin of replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than 3 kb; is from a mammal, including a primate; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the protein; or is a PCR primer, PCR product, or mutagenesis primer.

The invention provides also a cell or tissue comprising such a recombinant nucleic acid, particularly where the cell is: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell. A kit is also provided comprising the nucleic acid, and: a compartment comprising the nucleic acid; a compartment further comprising a chemokine protein or polypeptide; and/or instructions for use or disposal of reagents in the kit.

Particular embodiments include a nucleic acid which: hybridizes under wash conditions of 30° C and less than 2M salt to SEQ ID NO: 6 or 12; exhibits at least about 85% identity over a stretch of at least about 30 nucleotides to SEQ ID NO: 6 or 12; or, preferably, where the wash conditions are at 45° C and/or 500 mM salt; or the identity is at least 90% and/or the stretch is at least 55 nucleotides; and, even more preferably, the wash conditions are at 55° C and/or 150 mM salt; or the identity is at least 95% and/or the stretch is at least 75 nucleotides.

The invention also provides a method of modulating physiology or development of a cell or tissue culture cells comprising introducing into the cell an agonist or antagonist of a protein of SEQ ID NO: 6, 8, 12, or 14. Preferably, the physiology is attraction and the cell is from an immune system.

DETAILED DESCRIPTION

I. General

The present invention provides DNA sequences encoding mammalian proteins which exhibit structural properties or motifs characteristic of a cytokine or chemokine. For a review of the chemokine family, see, e.g., Lodi, et al. (1994) Science 263:1762-1767; Gronenborn and Clore (1991) Protein Engineering 4:263-269; Miller and Kranger (1992) Proc. Nat'l Acad. Sci. USA 89:2950-2954; Matsushima and Oppenheim (1989) Cytokine 1:2-13; Stoeckle and Baker (1990) New Biol. 2:313-323; Oppenheim, et al. (1991) Ann. Rev. Immunol. 9:617-648; Schall (1991) Cytokine 3:165-183; and The Cytokine Handbook Academic Press, NY.

The best characterized embodiments of this family of proteins were discovered from human and are designated human 61164 or 331D5 chemokines. The descriptions below are directed, for exemplary purposes, to primate embodiments, e.g., human, but are likewise applicable to related embodiments from other, e.g., natural, sources. These sources should include various vertebrates, typically warm

blooded animals, e.g., birds and mammals, particularly domestic animals, and primates.

The chemokine proteins of this invention are defined in part by their physicochemical and biological properties. The biological properties of the human chemokines described herein, e.g., human 61164 or 331D5, are defined, in fact, by their amino acid sequence, and mature size. They also should share biological properties with other similar chemokines. One of skill in the art will readily recognize that some sequence variations may be tolerated, e.g., conservative substitutions or positions remote from the central residues for receptor interaction or important tertiary structure features, without altering significantly the biological activity of the molecule. Conversely, non-conservative substitutions may be adapted to block selected functions.

These chemokines are present in specific tissue types, e.g., lymphoid tissues, and the interaction of the protein with a receptor will be important for mediating various aspects of cellular physiology or development. The cellular types which express messages encoding 61164 or 331D5 suggest that signals important in cell differentiation and development are mediated by them. See, e.g., Gilbert (1991) Developmental Biology (3d ed.) Sinauer Associates, Sunderland, MA; Browder, et al. (1991) Developmental Biology (3d ed.) Saunders, Philadelphia, PA.; Russo, et al. (1992) Development: The Molecular Genetic Approach Springer-Verlag, New York, N.Y.; and Wilkins (1993) Genetic Analysis of Animal Development (2d ed.) Wiley-Liss, New York, N.Y. Moreover, 61164 or 331D5 expression should serve to define certain cell subpopulations.

The 61164 chemokine producing profile of various cells is elucidated herein. 61164 exhibits significant identity to a chemokine-like domain within Gallid herpesvirus 1, a chicken virus implicated in Marek's disease, see, e.g., Peng, et al (1995) Virology 213:590-599. Using this domain the GENBANK EST (WashU-Merck EST project, St. Louis, MO) public database was searched. Two ESTs, T39765 and T29123, showed significant identity to this chemokine domain. The partial human 61163 nucleic acid sequence is set forth in SEQ ID NO

1. Predicted amino acid sequences from each reading frame are provided in SEQ ID NO: 2, 3 and 4. One phase translation of T39765 (SEQ ID NO: 2) yields the C-X-C chemokine motif "Cys-Arg-Cys" at positions 32-34). However, errors in sequencing probably exist, as this EST does not have a Met start codon. The error most likely occurs near Ser29, since it is within a conserved motif in the chemokine family. To further elucidate the correct sequence, 61164 was obtained from Research Genetics, Inc. (Huntsville, AL) I.M.A.G.E. Consortium cDNA clones. More reliable and complete sequences are provided in SEQ ID NO: 5 and 6. A mouse counterpart gene and predicted amino acid sequence are described in SEQ ID NO: 7 and 8. Cleavage of the mouse protein is most likely between Gly21 and Ile22.

Preliminary expression experiments indicate that 61164 is present in lymphoid tissue, i.e., at high levels in fetal spleen and tonsil mRNA. Lower expression was also detected in small intestine. Interestingly, this chemokine is not expressed in splenocytes. The tissue distribution suggests that the chemokine may be produced by B cells. Organ expression is consistent with a role in lymphocyte trafficking through secondary lymphoid organs. Moreover, the non-ELR CXC motif suggests that the molecule may have angiostatic properties, as with other non-ELR CXC chemokines. The R residue is probably important in receptor binding, and this chemokine may serve as a a receptor antagonist of other ELR chemokines. The angiostatic activity may slow down or block production of blood vessels at a localized site. Thus, administration of the chemokine at the site of a tumor may block the production of necessary vascularization to support growth of the tumor. Alternatively, wound healing may be modulated, as necessary, with the chemokine. Increased vascularization may lead to scar formation, but quick recovery; slower vascularization may prevent scar formation, e.g., in cosmetic or other surgery.

In addition, the SLR motif in the human form, which precedes the CXC, suggests the possibility that it may act as a natural receptor antagonist for ELR CXC chemokines.

331D5 was found by random sequencing of clones from a 90% CD1a+ cDNA library. The partial nucleic acid sequence and corresponding amino acid sequence are provided in SEQ ID NO: 9 and 10. The amino acid sequence does not contain an N-terminal methionine, which suggests that some sequence of the pre-chemokine molecule is absent, but sequence analysis further suggests that a signal sequence would correspond to a cleavage between about amino acids 15 and 16. Comparing homology with various ESTs deposited in GENBANK using the Sequencer 3.0 program (Gene Codes Corporation, Ann Arbor, MI) no matches were found at the nucleic acid level. Amino acid comparisons showed significant structural identity with CC chemokines, indicating that 331D5 may be a dendritic cell chemokine, or related thereto. SEQ ID NO: 11 and 12 provide the mature human 33105 nucleotide and amino acid sequences. Signal cleavage is probably somewhere between thr22 to gly25, probably between ala24 and gly25. A mouse counterpart is described in SEQ ID NO: 13 and 14. Signal cleavage is probably after ala24, but may be a few residues on either side.

The production of this chemokine by dendritic cells suggests a role in attracting naive B and/or T cells necessary to initiate an immune response, probably a T cell dependent response. Alternatively, the chemokine may exert an autocrine effect on dendritic cells themselves. Initiation of an immune response can be important, e.g., in a vaccine context. The chemokine may be useful, e.g., in recruitment of these important cell types, and may be included in, or supplied, with antigen for vaccine, e.g., with soluble antigen, or cellular antigen. Obvious contexts will be in vaccinations against infectious diseases, viruses, or perhaps even a tumor. The increased efficiency of immune response may decrease the number of injections necessary, or decrease the amount of antigen necessary. The chemokine may be used in combination with others, e.g., one or more of Dctactin (see Adema, et al. (1997) Nature 387:713-717), TECK (see Wang, et al., U.S.S.N. 08/887,977), MIP-1g (GenBank Z70296, also called CCF18), TARC (see Imai, et al. (1996) J. Biol. Chem. 271:21514-21519), and similar chemokines.

Alternatively, antagonists may be useful, e.g., in allergen administrations, alone or in combination with antagonists of the others.

II. Definitions

The term "binding composition" refers to molecules that bind with specificity to a 61164 or 331D5 chemokine, e.g., in an antibody-antigen interaction. However, other compounds, e.g., receptor proteins, may also specifically associate with 61164 or 331D5 chemokines to the exclusion of other molecules. Typically, the association will be in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent, and may include members of a multiprotein complex, including carrier compounds or dimerization partners. The molecule may be a polymer, or chemical reagent. No implication as to whether a 61164 or 331D5 chemokine is either the ligand or the receptor of a ligand-receptor interaction is necessarily represented, other than whether the interaction exhibits similar specificity, e.g., specific affinity. A functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule, e.g., which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists of the receptor, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press, Tarrytown, N.Y.

The term "binding agent:61164 or 331D5 chemokine protein complex", as used herein, refers to a complex of a binding agent and a 61164 or 331D5 chemokine protein that is formed by specific binding of the binding agent to the 61164 or 331D5 chemokine protein. Specific binding of the binding agent means that the binding agent has a specific binding site that recognizes a site on the 61164 or 331D5 chemokine protein. For example, antibodies raised to a 61164 or 331D5 chemokine protein and recognizing an epitope on the 61164 or 331D5 chemokine protein are capable of forming a binding agent:61164 or 331D5 chemokine protein complex by specific binding. Typically, the formation of a binding agent:61164

or 331D5 chemokine protein complex allows the measurement of 61164 or 331D5 chemokine protein in a mixture of other proteins and biologics. The term "antibody:61164 or 331D5 chemokine protein complex" refers to an embodiment in which the binding agent, e.g., is an antibody. The antibody may be monoclonal, polyclonal, or a binding fragment of an antibody, e.g, an Fab of F(ab)2 fragment. The antibody will preferably be a polyclonal antibody for cross-reactivity purposes.

"Homologous" nucleic acid sequences, when compared, exhibit significant similarity, or identity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison and/or phylogenetic relationship, or based upon hybridization conditions. Hybridization conditions are described in greater detail below.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other biologic components which naturally accompany a native sequence, e.g., proteins and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs, or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule. An isolated nucleic acid will usually contain homogeneous nucleic acid molecules, but will, in some embodiments, contain nucleic acids with minor sequence heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

As used herein, the term "61164 or 331D5 chemokine protein" shall encompass, when used in a protein context, a protein having amino acid sequences, particularly from the chemokine motif portions, shown in SEQ ID NO: 6, 8, 12, or 14, or a significant fragment of such a protein, preferably a natural embodiment. The invention also embraces a polypeptide which exhibits similar structure to human 61164 or 331D5 chemokine, e.g., which interacts with 61164 or 331D5

chemokine specific binding components. These binding components, e.g., antibodies, typically bind to a 61164 or 331D5 chemokine with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM.

The term "polypeptide" or "protein" as used herein includes a significant fragment or segment of chemokine motif portion of a 61164 or 331D5 chemokine, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids,more preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 35, 40, 45, 50, 60, 70, 80, etc. The invention encompasses proteins comprising a plurality of said segments.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, for example, products made by transforming cells with any non-naturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms.

Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar to fragments of these antigens, and fusions of sequences from various different species variants.

"Solubility" is reflected by sedimentation measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was classically performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge. See, Freifelder (1982) Physical Biochemistry (2d ed.) W.H. Freeman & Co., San Francisco, CA; and Cantor and Schimmel (1980) Biophysical Chemistry parts 1-3, W.H. Freeman & Co., San Francisco, CA. As a crude determination, a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10 minutes, and soluble molecules will remain in the supernatant. Asoluble particle or polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about 10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S. Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic

purposes, the temperature will usually be body temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner which approximates natural lipid bilayer interactions.

The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl)dimethylammonio]-1-propane sulfonate), or a low enough concentration as to avoid significant disruption of structural or physiological properties of the protein.

"Substantially pure" in a protein context typically means that the protein is isolated from other contaminating proteins, nucleic acids, and other biologicals derived from the original source organism. Purity, or "isolation" may be assayed by standard methods, and will ordinarily be at least about 50% pure, more ordinarily at least about 60% pure, generally at least about 70% pure, more generally at least about 80% pure, often at least about 85% pure, more often at least about 90% pure, preferably at least about 95% pure, more preferably at least about 98% pure, and in most preferred embodiments, at least 99% pure. Similar concepts apply, e.g., to antibodies or nucleic acids.

"Substantial similarity" in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least 56%, more generally at least 59%,

ordinarily at least 62%, more ordinarily at least 65%, often at least 68%, more often at least 71%, typically at least 74%, more typically at least 77%, usually at least 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides. Alternatively, substantial similarity exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence derived from SEQ ID NO: 1, 5, 7, 9, 11, or 13. Typically, selective hybridization will occur when there is at least about 55% similarity over a stretch of at least about 30 nucleotides, preferably at least about 65% over a stretch of at least about 25 nucleotides, more preferably at least about 75%, and most preferably at least about 90% over about 20 nucleotides. See Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of similarity comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides, e.g., 150, 200, etc.

"Stringent conditions", in referring to homology or substantial similarity in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. The combination of parameters is more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370. A nucleic acid probe which binds to a target nucleic acid under stringent conditions is specific for said target nucleic acid. Such a probe is typically more than 11 nucleotides in length, and is sufficiently identical or complementary to a target nucleic acid over the region specified by the sequence of the probe to bind the target under stringent hybridization conditions.

61164 or 331D5 chemokines from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. See, e.g., below. Similarity may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

The phrase "specifically binds to an antibody" or "specifically immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biological components. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not significantly bind other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to the human 61164 or 331D5 chemokine protein immunogen with the amino acid sequence depicted in SEQ ID NO: 6, 8, 12, or 14 can be selected to obtain antibodies specifically immunoreactive with 61164 or 331D5 chemokine proteins and not with other proteins. These antibodies recognize proteins highly similar to the homologous mouse 61164 or 331D5 chemokine protein.

III. Nucleic Acids

Human 61164 or 331D5 chemokine is exemplary of a larger class of structurally and functionally related proteins. These soluble chemokine proteins will serve to transmit signals between different cell types. The preferred embodiments, as disclosed, will be useful in standard procedures to isolate genes from different individuals or other species, e.g., warm blooded animals, such as birds and mammals. Cross hybridization will allow isolation of related genes encoding proteins from individuals, strains, or species. A number of different approaches are available to successfully isolate a suitable nucleic acid clone based upon

the information provided herein. Southern blot hybridization studies can qualitatively determine the presence of homologous genes in human, monkey, rat, mouse, dog, cow, and rabbit genomes under specific hybridization conditions.

Complementary sequences will also be used as probes or primers. Based upon identification of the likely amino terminus, other peptides should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

Techniques for nucleic acid manipulation of genes encoding 61164 or 331D5 chemokine proteins, such as subcloning nucleic acid sequences encoding polypeptides into expression vectors, labelling probes, DNA hybridization, and the like are described generally in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, which is incorporated herein by reference. This manual is hereinafter referred to as "Sambrook, et al."

There are various methods of isolating DNA sequences encoding 61164 or 331D5 chemokine proteins. For example, DNA is isolated from a genomic or cDNA library using labeled oligonucleotide probes having sequences identical or complementary to the sequences disclosed herein. Full-length probes may be used, or oligonucleotide probes may be generated by comparison of the sequences disclosed. Such probes can be used directly in hybridization assays to isolate DNA encoding 61164 or 331D5 chemokine proteins, or probes can be designed for use in amplification techniques such as PCR, for the isolation of DNA encoding 61164 or 331D5 chemokine proteins.

To prepare a cDNA library, mRNA is isolated from cells which expresses a 61164 or 331D5 chemokine protein. cDNA is prepared from the mRNA and ligated into a recombinant vector. The vector is transfected into a recombinant host for propagation, screening, and cloning. Methods for making and screening cDNA libraries are well known. See Gubler and Hoffman (1983) Gene 25:263-269 and Sambrook, et al.

For a genomic library, the DNA can be extracted from tissue and either mechanically sheared or enzymatically

digested to yield fragments of about 12-20 kb. The fragments are then separated by gradient centrifugation and cloned in bacteriophage lambda vectors. These vectors and phage are packaged in vitro, as described in Sambrook, et al. Recombinant phage are analyzed by plaque hybridization as described in Benton and Davis (1977) Science 196:180-182. Colony hybridization is carried out as generally described in e.g., Grunstein, et al. (1975) Proc. Natl. Acad. Sci. USA. 72:3961-3965.

DNA encoding a 61164 or 331D5 chemokine protein can be identified in either cDNA or genomic libraries by its ability to hybridize with the nucleic acid probes described herein, e.g., in colony or plaque hybridization assays. The corresponding DNA regions are isolated by standard methods familiar to those of skill in the art. See, e.g., Sambrook, et al.

Various methods of amplifying target sequences, such as the polymerase chain reaction, can also be used to prepare DNA encoding 61164 or 331D5 chemokine proteins. Polymerase chain reaction (PCR) technology is used to amplify such nucleic acid sequences directly from mRNA, from cDNA, and from genomic libraries or cDNA libraries. The isolated sequences encoding 61164 or 331D5 chemokine proteins may also be used as templates for PCR amplification.

Typically, in PCR techniques, oligonucleotide primers complementary to two 5' regions in the DNA region to be amplified are synthesized. The polymerase chain reaction is then carried out using the two primers. See Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, San Diego, CA. Primers can be selected to amplify the entire regions encoding a full-length 61164 or 331D5 chemokine protein or to amplify smaller DNA segments as desired. Once such regions are PCR-amplified, they can be sequenced and oligonucleotide probes can be prepared from sequence obtained using standard techniques. These probes can then be used to isolate DNA's encoding 61164 or 331D5 chemokine proteins.

Oligonucleotides for use as probes are usually chemically synthesized according to the solid phase

phosphoramidite triester method first described by Beaucage and Carruthers (1983) Tetrahedron Lett. 22(20):1859-1862, or using an automated synthesizer, as described in Needham-VanDevanter, et al. (1984) Nucleic Acids Res. 12:6159-6168. Purification of oligonucleotides is performed e.g., by native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson and Regnier (1983) J. Chrom. 255:137-149. The sequence of the synthetic oligonucleotide can be verified using, e.g., the chemical degradation method of Maxam, A.M. and Gilbert, W. in Grossman, L. and Moldave (eds.) (1980) Methods in Enzymology 65:499-560 Academic Press, New York.

An isolated nucleic acid encoding a human 61164 or 331D5 chemokine protein was identified. The nucleotide sequence and corresponding open reading frame are provided in SEQ ID NO: 1, 5, 6, 9, 10, 11, and 12. Correspondingly, mouse sequences were identified and nucleotide and corresponding open reading frame are provided as SEQ ID NO: 7, 8, 13, and 14.

These 61164 or 331D5 chemokines exhibit limited similarity to portions of chemokines. See, e.g., Matsushima and Oppenheim (1989) Cytokine 1:2-13; Oppenheim, et al. (1991) Ann. Rev. Immunol. 9:617-648; Schall (1991) Cytokine 3:165-183; and Gronenborn and Clore (1991) Protein Engineering 4:263-269. Other features of comparison are apparent between the 61164 or 331D5 chemokine and chemokine families. See, e.g., Lodi, et al. (1994) Science 263:1762-1766. In particular, β-sheet and α-helix residues can be determined using, e.g., RASMOL program, see Sayle and Milner-White (1995) TIBS 20:374-376; or Gronenberg, et al. (1991) Protein Engineering 4:263-269; and other structural features are defined in Lodi, et al. (1994) Science 263:1762-1767. These secondary and tertiary features assist in defining further the C, CC, and CXC structural features, along with spacing of appropriate cysteine residues.

This invention provides isolated DNA or fragments to encode a 61164 or 331D5 chemokine protein. In addition, this invention provides isolated or recombinant DNA which encodes a protein or polypeptide which is capable of hybridizing

under appropriate conditions, e.g., high stringency, with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact ligand, or fragment, and have an amino acid sequence as disclosed in SEQ ID NO: 6, 8, 12, or 14, particularly natural embodiments. Preferred embodiments will be full length natural sequences, from isolates, e.g., about 11,000 to 12,500 daltons in size when unglycosylated, or fragments of at least about 6,000 daltons, more preferably at least about 8,000 daltons. In glycosylated form, the protein may exceed 12,500 daltons. Further, this invention contemplates the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which are homologous to a 61164 or 331D5 chemokine protein or which were isolated using cDNA encoding a 61164 or 331D5 chemokine protein as a probe. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others. Also embraced are methods for making expression vectors with these sequences, or for making, e.g., expressing and purifying, protein products.

IV. Making 61164 or 331D5 chemokines

DNAs which encode a 61164 or 331D5 chemokine or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a wide variety of cell lines or tissue samples. Methods for doing so, or making expression vectors are described herein.

These DNAs can be expressed in a wide variety of host cells for the synthesis of a full-length protein or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies. Each 61164 or 331D5 chemokine or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially purified to be free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are

particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The antigen, e.g., 61164 or 331D5 chemokine, or portions thereof, may be expressed as fusions with other proteins or possessing an epitope tag.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired antigen gene or its fragments, usually operably linked to appropriate genetic control elements that are recognized in a suitable host cell. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently from the host cell.

The vectors of this invention contain DNAs which encode a 61164 or 331D5 chemokine, or a fragment thereof, typically encoding, e.g., a biologically active polypeptide, or protein. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a 61164 or 331D5 chemokine protein in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the protein is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the protein or its fragments in various hosts

using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of a 61164 or 331D5 chemokine gene or its fragments into the host DNA by recombination, or to integrate a promoter which controls expression of an endogenous gene.

Vectors, as used herein, contemplate plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector, but many other forms of vectors which serve an equivalent function are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual Elsevier, N.Y.; and Rodriquez, et al. (eds.) (1988) Vectors: A Survey of Molecular Cloning Vectors and Their Uses Buttersworth, Boston, MA.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or its derivatives. Vectors that can be used to express 61164 or 331D5 chemokines or 61164 or 331D5 chemokine fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing

Lambda-, trp-, lac-, and Ipp-derived Promoters", in Rodriguez and Denhardt (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses 10:205-236 Buttersworth, Boston, MA.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with 61164 or 331D5 chemokine sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used generically to represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the desired protein or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are typically the preferred host cells for expression of the functionally active 61164 or 331D5 chemokine protein. In principle, many higher eukaryotic tissue culture cell lines may be used, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred to achieve proper processing, both cotranslationally and posttranslationally. Transformation or transfection and propagation of such cells is routine. Useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (e.g., if genomic DNA is used), a polyadenylation site, and a transcription termination site.

These vectors also may contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

It is likely that 61164 or 331D5 chemokines need not be glycosylated to elicit biological responses. However, it will occasionally be desirable to express a 61164 or 331D5 chemokine polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., in unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the 61164 or 331D5 chemokine gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. It is further understood that over glycosylation may be detrimental to 61164 or 331D5 chemokine biological activity, and that one of skill may perform routine testing to optimize the degree of glycosylation which confers optimal biological activity.

A 61164 or 331D5 chemokine, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochem. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that 61164 or 331D5 chemokines have been characterized, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides.

These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis Springer-Verlag, New York, NY; and Bodanszky (1984) The Principles of Peptide Synthesis Springer-Verlag, New York, NY. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes.

The prepared protein and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, for example, by extraction, precipitation, electrophoresis and various forms of chromatography, and the like. The 61164 or 331D5 chemokines of this invention can be obtained in varying degrees of purity depending upon its desired use. Purification can be accomplished by use of known protein purification techniques or by the use of the antibodies or binding partners herein described, e.g., in immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate source cells, lysates of other cells expressing the ligand, or lysates or supernatants of cells producing the 61164 or 331D5 chemokines as a result of recombinant DNA techniques, see below.

Multiple cell lines may be screened for one which expresses a 61164 or 331D5 chemokine at a high level compared with other cells. Various cell lines, e.g., a mouse thymic stromal cell line TA4, is screened and selected for its favorable handling properties. Natural 61164 or 331D5 chemokines can be isolated from natural sources, or by expression from a transformed cell using an appropriate expression vector. Purification of the expressed protein is

achieved by standard procedures, or may be combined with engineered means for effective purification at high efficiency from cell lysates or supernatants. Epitope or other tags, e.g., FLAG or His_6 segments, can be used for such purification features.

V. Antibodies

Antibodies can be raised to various 61164 or 331D5 chemokines, including individual, polymorphic, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in their recombinant forms. Additionally, antibodies can be raised to 61164 or 331D5 chemokines in either their active forms or in their inactive forms. Anti-idiotypic antibodies may also be used.

A. Antibody Production

A number of immunogens may be used to produce antibodies specifically reactive with 61164 or 331D5 chemokine proteins. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein may also be used either in pure or impure form. Synthetic peptides, made using the human or mouse 61164 or 331D5 chemokine protein sequences described herein, may also used as an immunogen for the production of antibodies to 61164 or 331D5 chemokines. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described herein, and purified as described. Naturally folded or denatured material can be used, as appropriate, for producing antibodies. Either monoclonal or polyclonal antibodies may be generated for subsequent use in immunoassays to measure the protein.

Methods of producing polyclonal antibodies are known to those of skill in the art. Typically, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the 61164 or 331D5 chemokine protein of interest. When appropriately high titers of antibody to the immunogen are

obtained, usually after repeated immunizations, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired. See, e.g., Harlow and Lane; or Coligan.

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (see, Kohler and Milstein (1976) Eur. J. Immunol. 6:511-519, incorporated herein by reference). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according, e.g., to the general protocol outlined by Huse, et al. (1989) Science 246:1275-1281.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of 61164 or 331D5 chemokines can be raised by immunization of animals with conjugates of the fragments with carrier proteins as described above. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective 61164 or 331D5 chemokines, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μM, typically at least about 10 μM, more typically at least about 30 μM, preferably at least about 10 μM, and more preferably at least about 3 μM or better.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice,

rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY; and particularly in Kohler and Milstein (1975) Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve selection of libraries of antibodies in phage or similar vectors. See, e.g., Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Also, recombinant immunoglobulins may be produced. See, Cabilly, U.S. Patent No. 4,816,567; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033.

The antibodies of this invention are useful for affinity chromatography in isolating 61164 or 331D5 chemokine protein. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, SEPHADEX, or the like, where a cell lysate or supernatant may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby purified 61164 or 331D5 chemokine protein will be released.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies to 61164 or 331D5 chemokines may be used for the identification of cell populations expressing 61164 or 331D5 chemokines. By assaying the expression products of cells expressing 61164 or 331D5 chemokines it is possible to diagnose disease, e.g., immune-compromised conditions.

Antibodies raised against each 61164 or 331D5 chemokine will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

B. Immunoassays

A particular protein can be measured by a variety of immunoassay methods. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds.) (1991) Basic and Clinical Immunology (7th ed.). Moreover, the immunoassays of the present invention can be performed in many configurations, which are reviewed extensively in Maggio (ed.) (1980) Enzyme Immunoassay CRC Press, Boca Raton, Florida; Tijan (1985) "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B.V., Amsterdam; and Harlow and Lane Antibodies, A Laboratory Manual, supra, each of which is incorporated herein by

reference. See also Chan (ed.) (1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) (1991) Principles and Practice of Immunoassays Stockton Press, NY; and Ngo (ed.) (1988) Non-isotopic Immunoassays Plenum Press, NY.

Immunoassays for measurement of 61164 or 331D5 chemokine proteins can be performed by a variety of methods known to those skilled in the art. In brief, immunoassays to measure the protein can be either competitive or noncompetitive binding assays. In competitive binding assays, the sample to be analyzed competes with a labeled analyte for specific binding sites on a capture agent bound to a solid surface. Preferably the capture agent is an antibody specifically reactive with 61164 or 331D5 chemokine proteins produced as described above. The concentration of labeled analyte bound to the capture agent is inversely proportional to the amount of free analyte present in the sample.

In a competitive binding immunoassay, the 61164 or 331D5 chemokine protein present in the sample competes with labeled protein for binding to a specific binding agent, for example, an antibody specifically reactive with the 61164 or 331D5 chemokine protein. The binding agent may be bound to a solid surface to effect separation of bound labeled protein from the unbound labeled protein. Alternately, the competitive binding assay may be conducted in liquid phase and a variety of techniques known in the art may be used to separate the bound labelled protein from the unbound labeled protein. Following separation, the amount of bound labeled protein is determined. The amount of protein present in the sample is inversely proportional to the amount of labeled protein binding.

Alternatively, a homogeneous immunoassay may be performed in which a separation step is not needed. In these immunoassays, the label on the protein is altered by the binding of the protein to its specific binding agent. This alteration in the labeled protein results in a decrease or increase in the signal emitted by label, so that measurement of the label at the end of the immunoassay allows for detection or quantitation of the protein.

61164 or 331D5 chemokine proteins may also be determined by a variety of noncompetitive immunoassay methods. For example, a two-site, solid phase sandwich immunoassay may be used. In this type of assay, a binding agent for the protein, for example an antibody, is attached to a solid support. A second protein binding agent, which may also be an antibody, and which binds the protein at a different site, is labelled. After binding at both sites on the protein has occurred, the unbound labeled binding agent is removed and the amount of labeled binding agent bound to the solid phase is measured. The amount of labeled binding agent bound is directly proportional to the amount of protein in the sample.

Western blot analysis can be used to determine the presence of 61164 or 331D5 chemokine proteins in a sample. Electrophoresis is carried out, for example, on a tissue sample suspected of containing the protein. Following electrophoresis to separate the proteins, and transfer of the proteins to a suitable solid support, e.g., a nitrocellulose filter, the solid support is incubated with an antibody reactive with the protein. This antibody may be labeled, or alternatively may be detected by subsequent incubation with a second labeled antibody that binds the primary antibody.

The immunoassay formats described above employ labeled assay components. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A wide variety of labels and methods may be used. Traditionally, a radioactive label incorporating ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P was used. Non-radioactive labels include ligands which bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. The choice of label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation. For a review of various labelling or signal producing systems which may be used, see U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Antibodies reactive with a particular protein can also be measured by a variety of immunoassay methods. For a review of immunological and immunoassay procedures applicable to the measurement of antibodies by immunoassay techniques, see Stites and Terr (eds.) Basic and Clinical Immunology (7th ed.) supra; Maggio (ed.) Enzyme Immunoassay, supra; and Harlow and Lane Antibodies, A Laboratory Manual, supra.

In brief, immunoassays to measure antisera reactive with 61164 or 331D5 chemokine proteins can be either competitive or noncompetitive binding assays. In competitive binding assays, the sample analyte competes with a labeled analyte for specific binding sites on a capture agent bound to a solid surface. Preferably the capture agent is a purified recombinant 61164 or 331D5 chemokine protein produced as described above. Other sources of 61164 or 331D5 chemokine proteins, including isolated or partially purified naturally occurring protein, may also be used. Noncompetitive assays include sandwich assays, in which the sample analyte is bound between two analyte-specific binding reagents. One of the binding agents is used as a capture agent and is bound to a solid surface. The second binding agent is labeled and is used to measure or detect the resultant complex by visual or instrument means. A number of combinations of capture agent and labelled binding agent can be used. A variety of different immunoassay formats, separation techniques, and labels can be also be used similar to those described above for the measurement of 61164 or 331D5 chemokine proteins.

VI. Purified 61164 or 331D5 chemokines

Human 61164 or 331D5 chemokine amino acid sequences are provided in SEQ ID NO: 6 and 12. Mouse nucleotide and amino acid sequences are provided in SEQ ID NO: 7, 8, 13, and 14.

Purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be presented to an immune system to generate polyclonal and monoclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor

Press, NY, which are incorporated herein by reference. Alternatively, a 61164 or 331D5 chemokine receptor can be useful as a specific binding reagent, and advantage can be taken of its specificity of binding, for, e.g., purification of a 61164 or 331D5 chemokine ligand.

The specific binding composition can be used for screening an expression library made from a cell line which expresses a 61164 or 331D5 chemokine. Many methods for screening are available, e.g., standard staining of surface expressed ligand, or by panning. Screening of intracellular expression can also be performed by various staining or immunofluorescence procedures. The binding compositions could be used to affinity purify or sort out cells expressing the ligand.

The peptide segments, along with comparison to homologous genes, can also be used to produce appropriate oligonucleotides to screen a library. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting desired clones from a library, including natural allelic and polymorphic variants.

The peptide sequences allow preparation of peptides to generate antibodies to recognize such segments, and allow preparation of oligonucleotides which encode such sequences. The sequence also allows for synthetic preparation, e.g., see Dawson, et al. (1994) Science 266:776-779. Since 61164 or 331D5 chemokines may be secreted proteins, the gene will normally possess an N-terminal signal sequence, which is removed upon processing and secretion. Analysis of the structural features in comparison with the most closely related reported sequences has revealed similarities with other cytokines, particularly the class of proteins known as CC and CXC chemokines.

VII. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence similarity with an amino acid sequence of a 61164 or 331D5 chemokine. Natural

variants include individual, polymorphic, allelic, strain, or species variants.

Amino acid sequence similarity, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences include natural polymorphic, allelic, and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 50-100% similarity (if gaps can be introduced), to 75-100% similarity (if conservative substitutions are included) with the amino acid sequence of the 61164 or 331D5 chemokine. Similarity measures will be at least about 50%, generally at least 60%, more generally at least 65%, usually at least 70%, more usually at least 75%, preferably at least 80%, and more preferably at least 80%, and in particularly preferred embodiments, at least 85% or more. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison Chapter One, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI.

Nucleic acids encoding mammalian 61164 or 331D5 chemokine proteins will typically hybridize to the nucleic acid sequence of SEQ ID NO: 1, 5, 7, 11, or 13 under stringent conditions. For example, nucleic acids encoding human 61164 or 331D5 chemokine proteins will normally hybridize to the nucleic acid of SEQ ID NO: 1, 5, 9, or 11 under stringent hybridization conditions. Generally, stringent conditions are selected to be about 10° C lower than the thermal melting point (Tm) for the probe sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the

target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is about 0.2 molar at pH 7 and the temperature is at least about 50° C. Other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents such as formamide, and the extent of base mismatching. A preferred embodiment will include nucleic acids which will bind to disclosed sequences in 50% formamide and 200 mM NaCl at 42° C.

An isolated 61164 or 331D5 chemokine DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and short inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode 61164 or 331D5 chemokine antigens, their derivatives, or proteins having highly similar physiological, immunogenic, or antigenic activity.

Modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant 61164 or 331D5 chemokine derivatives include predetermined or site-specific mutations of the respective protein or its fragments. "Mutant 61164 or 331D5 chemokine" encompasses a polypeptide otherwise falling within the homology definition of the human 61164 or 331D5 chemokine as set forth above, but having an amino acid sequence which differs from that of a 61164 or 331D5 chemokine as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant 61164 or 331D5 chemokine" generally includes proteins having significant similarity with a protein having a sequence of SEQ ID NO: 6, 8, 12, or 14, e.g., natural embodiments, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most or all of the disclosed sequence. This applies also to polymorphic variants from different individuals. Similar concepts apply to different 61164 or 331D5 chemokine proteins, particularly

those found in various warm blooded animals, e.g., mammals and birds. As stated before, it is emphasized that descriptions are generally meant to encompass other 61164 or 331D5 chemokine proteins, not limited to the human or mouse embodiments specifically discussed.

Although site specific mutation sites are predetermined, mutants need not be site specific. 61164 or 331D5 chemokine mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxyl- terminal fusions, e.g. epitope tags. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See also, Sambrook, et al. (1989) and Ausubel, et al. (1987 and Supplements). The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a 61164 or 331D5 chemokine polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, protein-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd,

et al. (1988) J. Biol. Chem. 263:15985-15992. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of protein-binding specificities and other functional domains.

VIII. Binding Agent:61164 or 331D5 chemokine Protein Complexes

A 61164 or 331D5 chemokine protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ ID NO: 6, 8, 12, or 14, is typically determined in an immunoassay. The immunoassay uses a polyclonal antiserum which was raised to a protein of SEQ ID NO: 6, 8, 12, or 14. This antiserum is selected to have low crossreactivity against other chemokines and any such crossreactivity is removed by immunoabsorbtion prior to use in the immunoassay.

In order to produce antisera for use in an immunoassay, the protein of SEQ ID NO: 6, 8, 12, or 14, is isolated as described herein. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice such as balb/c is immunized with the protein of SEQ ID NO: 6, 8, 12, or 14, using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra). Alternatively, a synthetic peptide, preferably near full length, derived from the sequences disclosed herein and conjugated to a carrier protein can be used an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against C, C-C, and CXC chemokines, using a competitive binding immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Preferably two chemokines are used in this determination in conjunction with human 61164 or 331D5 chemokine.

Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, a

protein of SEQ ID NO: 6, 8, 12, or 14 can be immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the protein of SEQ ID NO: 6 and/or 8; or 12 and/or 14. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorbtion with the above-listed proteins.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein (e.g., the 61164 or 331D5 chemokine chemokine motif of SEQ ID NO: 6, 8, 12, or 14). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than twice the amount of the protein, e.g., of SEQ ID NO: 6 and/or 8 that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.

It is understood that each of 61164 or 331D5 chemokine proteins are members of respective families of homologous proteins that comprise two or more genes. For a particular gene product, such as the human 61164 or 331D5 chemokine protein, the term refers not only to the amino acid sequences disclosed herein, but also to other proteins that are polymorphic, allelic, non-allelic, or species variants. It is also understood that the term "human 61164 or 331D5 chemokine" includes nonnatural mutations introduced by deliberate mutation using conventional recombinant technology such as single site mutation, or by excising short sections of DNA encoding 61164 or 331D5 chemokine proteins, or by substituting new amino acids, or adding new amino acids. Such minor alterations should substantially maintain the

immunoidentity of the original molecule and/or its biological activity. Thus, these alterations include proteins that are specifically immunoreactive with a designated naturally occurring 61164 or 331D5 chemokine protein, for example, the human 61164 or 331D5 chemokine protein shown in SEQ ID NO: 6 or 12. The biological properties of the altered proteins can be determined by expressing the protein in an appropriate cell line and measuring, e.g., a chemotactic effect. Particular protein modifications considered minor would include conservative substitution of amino acids with similar chemical properties, as described above for the 61164 or 331D5 chemokine as a whole. By aligning a protein optimally with the protein of SEQ ID NO: 6 and/or 8; or 12 and/or 14, and by using the conventional immunoassays described herein to determine immunoidentity, or by using lymphocyte chemotaxis assays, one can determine the protein compositions of the invention.

IX. Functional Variants

The blocking of physiological response to 61164 or 331D5 chemokine may result from the inhibition of binding of the protein to its receptor, e.g., through competitive inhibition. Thus, in vitro assays of the present invention will often use isolated protein, membranes from cells expressing a recombinant membrane associated 61164 or 331D5 chemokine, soluble fragments comprising receptor binding segments of these proteins, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or protein mutations and modifications, e.g., protein analogs. This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to antigen or receptor fragments compete with a test compound for binding to the protein. Small chemical drug libraries may also be passed through automated screening assays. In this manner, the antibodies can be used to detect the presence of a polypeptide which shares one or more antigenic binding sites

of the protein and can also be used to occupy binding sites on the protein that might otherwise interact with a receptor.

"Derivatives" of 61164 or 331D5 chemokine antigens include amino acid sequence mutants, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in 61164 or 331D5 chemokine amino acid side chains or at the N- or C- termini, by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species. Covalent attachment to carrier proteins may be important when immunogenic moieties are haptens.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine, or other moieties, including ribosyl groups or cross-linking reagents.

A major group of derivatives are covalent conjugates of the 61164 or 331D5 chemokine or fragments thereof with other proteins or polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred protein derivatization sites with cross-

linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between 61164 or 331D5 chemokine and other homologous or heterologous proteins are also provided. Many growth factors and cytokines are homodimeric entities, and a repeat construct may have various advantages, including lessened susceptibility to proteolytic degradation. Moreover, many receptors require dimerization to transduce a signal, and various dimeric proteins or domain repeats can be desirable. Heterologous polypeptides may be fusions between different surface markers, resulting in, e.g., a hybrid protein exhibiting receptor binding specificity. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a protein, e.g., a receptor-binding segment, so that the presence or location of the fused protein may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial β-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

This invention also contemplates the use of derivatives of 61164 or 331D5 chemokine other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into the three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in

purification methods such as for affinity purification of ligands or other binding ligands. For example, a 61164 or 331D5 chemokine antigen can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-61164 or 331D5 chemokine antibodies or its receptor. The 61164 or 331D5 chemokine can also be labeled with a detectable group, e.g., radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays. Purification of 61164 or 331D5 chemokines may be effected by immobilized antibodies or receptor.

Isolated 61164 or 331D5 chemokine genes will allow transformation of cells lacking expression of corresponding 61164 or 331D5 chemokine, e.g., either species types or cells which lack corresponding proteins and exhibit negative background activity. Expression of transformed genes will allow isolation of antigenically pure cell lines, with defined or single specie variants. This approach will allow for more sensitive detection and discrimination of the physiological effects of 61164 or 331D5 chemokine receptor proteins. Subcellular fragments, e.g., cytoplasts or membrane fragments, can be isolated and used.

X. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in the general description for developmental abnormalities, or below in the description of kits for diagnosis.

61164 or 331D5 chemokine nucleotides, e.g., human 61164 or 331D5 chemokine DNA or RNA, may be used as a component in a forensic assay. For instance, the nucleotide sequences provided may be labeled using, e.g., ^{32}P or biotin and used to probe standard restriction fragment polymorphism blots, providing a measurable character to aid in distinguishing

between individuals. Such probes may be used in well-known forensic techniques such as genetic fingerprinting. In addition, nucleotide probes made from 61164 or 331D5 chemokine sequences may be used in in situ assays to detect chromosomal abnormalities. For instance, rearrangements in the human chromosome encoding a 61164 or 331D5 chemokine gene may be detected via well-known in situ techniques, using 61164 or 331D5 chemokine probes in conjunction with other known chromosome markers.

Antibodies and other binding agents directed towards 61164 or 331D5 chemokine proteins or nucleic acids may be used to purify the corresponding 61164 or 331D5 chemokine molecule. As described in the Examples below, antibody purification of 61164 or 331D5 chemokine components is both possible and practicable. Antibodies and other binding agents may also be used in a diagnostic fashion to determine whether 61164 or 331D5 chemokine components are present in a tissue sample or cell population using well-known techniques described herein. The ability to attach a binding agent to a 61164 or 331D5 chemokine provides a means to diagnose disorders associated with 61164 or 331D5 chemokine misregulation. Antibodies and other 61164 or 331D5 chemokine binding agents may also be useful as histological markers. As described in the examples below, 61164 or 331D5 chemokine expression is limited to specific tissue types. By directing a probe, such as an antibody or nucleic acid to a 61164 or 331D5 chemokine it is possible to use the probe to distinguish tissue and cell types in situ or in vitro.

This invention also provides reagents with significant therapeutic value. The 61164 or 331D5 chemokine (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to a 61164 or 331D5 chemokine, are useful in the treatment of conditions associated with abnormal physiology or development, including abnormal proliferation, e.g., cancerous conditions, or degenerative conditions. Abnormal proliferation, regeneration, degeneration, and atrophy may be modulated by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or

disorder associated with abnormal expression or abnormal signaling by a 61164 or 331D5 chemokine is a target for an agonist or antagonist of the protein. The proteins likely play a role in regulation or development of neuronal or hematopoietic cells, e.g., lymphoid cells, which affect immunological responses.

Other abnormal developmental conditions are known in cell types shown to possess 61164 or 331D5 chemokine mRNA by northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, NJ; and Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, NY. Developmental or functional abnormalities, e.g., of the neuronal or immune system, cause significant medical abnormalities and conditions which may be susceptible to prevention or treatment using compositions provided herein.

Certain chemokines have also been implicated in viral replication mechanisms. See, e.g., Cohen (1996) Science 272:809-810; Feng, et al. (1996) Science 272:872-877; and Cocchi, et al. (1995) Science 270:1811-1816. The 61164 or 331D5 chemokine may be useful in a similar context. Alternatively, the stalk structure may be very important in presentation of the ligand domain, and other chemokines may be advantageously substituted for the chemokine domain in this molecule.

Recombinant 61164 or 331D5 chemokine or 61164 or 331D5 chemokine antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using antibodies or receptor or fragments thereof can identify compounds having binding affinity to 61164 or 331D5 chemokine, including isolation of associated

components. Assays for compounds which can block chemokine-receptor signaling may be developed. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the protein. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of a 61164 or 331D5 chemokine. This invention further contemplates the therapeutic use of antibodies to 61164 or 331D5 chemokine as antagonists. This approach should be particularly useful with other 61164 or 331D5 chemokine species variants.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, NJ. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 µM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

61164 or 331D5 chemokines, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosgae Forms: Tablets Dekker, NY; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other therapeutic agents.

Both the naturally occurring and the recombinant forms of the 61164 or 331D5 chemokines of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, and other descriptions of chemical diversity libraries, which describe

means for testing of binding affinity by a plurality of compounds. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble 61164 or 331D5 chemokine as provided by this invention.

For example, antagonists can normally be found once the protein has been structurally defined. Testing of potential protein analogs is now possible upon the development of highly automated assay methods using a purified receptor. In particular, new agonists and antagonists will be discovered by using screening techniques described herein. Of particular importance are compounds found to have a combined binding affinity for multiple 61164 or 331D5 chemokine receptors, e.g., compounds which can serve as antagonists for species variants of a 61164 or 331D5 chemokine. Compound library screening will elucidate structures which may antagonize chemokine-receptor interactions.

This invention is particularly useful for screening compounds by using recombinant protein in a variety of drug screening techniques. The advantages of using a recombinant protein in screening for specific ligands include: (a) improved renewable source of the 61164 or 331D5 chemokine from a specific source; (b) potentially greater number of ligands per cell giving better signal to noise ratio in assays; and (c) species variant specificity (theoretically giving greater biological and disease specificity) See, e.g. Lam, 1997, Anticancer Drug Dis. 12:145-167; Salmon, et al., 1996, Mol. Diversity 2:57-63; Salmon et al., 1994, ACTA Oncol. 33:127-131.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing a 61164 or 331D5 chemokine receptor. Cells may be isolated which express a receptor in isolation from any others. Such cells, either in viable or fixed form, can be used for standard ligand/receptor binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses. Competitive assays are

particularly useful, where the cells (source of 61164 or 331D5 chemokine) are contacted and incubated with a labeled receptor or antibody having known binding affinity to the ligand, such as ^{125}I-antibody, and a test sample whose binding affinity to the binding composition is being measured. The bound and free labeled binding compositions are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled receptor binding to the known source. Any one of numerous techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on 61164 or 331D5 chemokine mediated functions, e.g., second messenger levels, i.e., Ca^{++}; cell proliferation; inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

Another method utilizes membranes from transformed eukaryotic or prokaryotic host cells as the source of a 61164 or 331D5 chemokine. These cells are stably transformed with DNA vectors directing the expression of a 61164 or 331D5 chemokine, e.g., an engineered membrane bound form. Essentially, the membranes would be prepared from the cells and used in a receptor/ligand binding assay such as the competitive assay set forth above.

Still another approach is to use solubilized, unpurified or solubilized, purified 61164 or 331D5 chemokine from transformed eukaryotic or prokaryotic host cells. This allows for a "molecular" binding assay with the advantages of increased specificity, the ability to automate, and high drug test throughput.

Another technique for drug screening involves an approach which provides high throughput screening for

compounds having suitable binding affinity to a 61164 or 331D5 chemokine antibody and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al., supra. Then all the pins are reacted with solubilized, unpurified or solubilized, purified 61164 or 331D5 chemokine antibody, and washed. The next step involves detecting bound 61164 or 331D5 chemokine antibody.

Rational drug design may also be based upon structural studies of the molecular shapes of the 61164 or 331D5 chemokine and other effectors or analogs. See, e.g., Methods in Enzymology vols 202 and 203. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography Academic Press, NY.

A purified 61164 or 331D5 chemokine can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to these ligands can be used as capture antibodies to immobilize the respective ligand on the solid phase.

XI. Kits

This invention also contemplates use of 61164 or 331D5 chemokine proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of 61164 or 331D5 chemokine or a 61164 or 331D5 chemokine receptor. Typically the kit will have a compartment containing either a defined 61164 or 331D5 chemokine peptide or gene segment or a reagent which

recognizes one or the other, e.g., receptor fragments or antibodies.

A kit for determining the binding affinity of a test compound to a 61164 or 331D5 chemokine would typically comprise a test compound; a labeled compound, e.g., a receptor or antibody having known binding affinity for the 61164 or 331D5 chemokine; a source of 61164 or 331D5 chemokine (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the 61164 or 331D5 chemokine. Once compounds are screened, those having suitable binding affinity to the 61164 or 331D5 chemokine can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the receptor. The availability of recombinant 61164 or 331D5 chemokine polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, for example, a 61164 or 331D5 chemokine in a sample would typically comprise a labeled compound, e.g., receptor or antibody, having known binding affinity for the 61164 or 331D5 chemokine, a source of 61164 or 331D5 chemokine (naturally occurring or recombinant), and a means for separating the bound from free labeled compound, for example, a solid phase for immobilizing the 61164 or 331D5 chemokine. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for the 61164 or 331D5 chemokine or ligand fragments are useful in diagnostic applications to detect the presence of elevated levels of 61164 or 331D5 chemokine and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the ligand in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-61164 or 331D5 chemokine complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked

immunosorbentassay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to a 61164 or 331D5 chemokine or to a particular fragment thereof. Similar assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY; Chan (ed.) (1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) (1991) Principles and Practice of Immunoassay Stockton Press, NY; and Ngo (ed.) (1988) Nonisotopic Immunoassay Plenum Press, NY.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against a 61164 or 331D5 chemokine, as such may be diagnostic of various abnormal states. For example, overproduction of 61164 or 331D5 chemokine may result in production of various immunological or other medical reactions which may be diagnostic of abnormal physiological states, e.g., in cell growth, acitivation, or differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or receptor, or labeled 61164 or 331D5 chemokine is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification, or may be modified in a variety of ways. For

example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the protein, test compound, 61164 or 331D5 chemokine, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the free test compound. The 61164 or 331D5 chemokine can be immobilized on various matrices followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the 61164 or 331D5 chemokine to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of ligand/receptor or ligand/antibody complex by any of several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such

as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of a 61164 or 331D5 chemokine. These sequences can be used as probes for detecting levels of the 61164 or 331D5 chemokine message in samples from natural sources, or patients suspected of having an abnormal condition, e.g., cancer or developmental problem. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several kilobases. Various labels may be employed, most commonly radionuclides, particularly ^{32}P. However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorophores, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out using many conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of

multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

XII. Receptor Isolation

Having isolated a binding partner of a specific interaction, methods exist for isolating the counter-partner. See, Gearing, et al. (1989) EMBO J. 8:3667-3676. For example, means to label a 61164 or 331D5 chemokine without interfering with the binding to its receptor can be determined. For example, an affinity label or epitope tag can be fused to either the amino- or carboxyl-terminus of the ligand. An expression library can be screened for specific binding of the 61164 or 331D5 chemokine, e.g., by cell sorting, or other screening to detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:11267-11271. Alternatively, a panning method may be used. See, e.g., Seed and Aruffo (1987) Proc. Nat'l Acad. Sci. USA 84:3365-3369. A two-hybrid slection system may also be applied making appropriate constructs with the available chemokine sequences. See, e.g., Fields and Song (1989) Nature 340:245-246.

Protein cross-linking techniques with label can be applied to isolate binding partners of a 61164 or 331D5 chemokine. This would allow identification of proteins which specifically interact with a 61164 or 331D5 chemokine, e.g., in a ligand-receptor like manner. Typically, the chemokine family binds to receptors of the seven transmembrane receptor family, and the receptor for the 61164 or 331D5 chemokine is likely to exhibit a similar structure. Thus, it is likely that the receptor will be found by expression in a system which is capable of expressing such a membrane protein in a form capable of exhibiting ligand binding capability.

Orphan G-protein coupled receptors may be tested for calcium clux response to binding with these ligands.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

EXAMPLES

I. General Methods

Many of the standard methods below are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.) Vols. 1-3, CSH Press, NY; Ausubel, et al., Biology Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology Wiley/Greene, NY; Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, NY. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification," Methods in Enzymology vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, NJ, or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments (epitope tags), e.g., to a FLAG sequence or an equivalent which can be fused, e.g., via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, NY; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Standard immunological techniques are described, e.g., in Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. Assays for monocyte adhesion are described, e.g., in McEvoy, et al: (1997) J. Exp. Med. 185:2069-2077, and references cited therein. Angiogenic assays are described, e.g., in

Yanagisawa-Miwa, et al. (1992) Science 257:1401-1403; and Padua, et al. (1995) Mol. Cell. Biochem. 143:129-135. Assays for neural cell biological activities are described, e.g., in Wouterlood (ed. 1995) Neuroscience Protocols modules 10, Elsevier; Methods in Neurosciences Academic Press; and Neuromethods Humana Press, Totowa, NJ. Methodology of developmental systems is described, e.g., in Meisami (ed.) Handbook of Human Growth and Developmental Biology CRC Press; and Chrispeels (ed.) Molecular Techniques and Approaches in Developmental Biology Interscience.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

II. Isolation of human 61164 or 331D5 chemokine clone

A clone encoding the human 61164 or 331D5 chemokine is isolated from a natural source by many different possible methods. Given the sequences provided herein, PCR primers or hybridization probes are selected and/or constructed to isolate either genomic DNA segments or cDNA reverse transcripts. Appropriate cell sources include human tissues, e.g., brain libraries. Tissue distribution below also suggests source tissues. Genetic and polymorphic or allelic variants are isolated by screening a population of individuals.

PCR based detection is performed by standard methods, preferably using primers from opposite ends of the coding sequence, but flanking segments might be selected for specific purposes.

Alternatively, hybridization probes are selected. Particular AT or GC contents of probes are selected depending upon the expected homology and mismatching expected. Appropriate stringency conditions are selected to balance an appropriate positive signal to background ratio. Successive washing steps are used to collect clones of greater homology.

Further clones are isolated using an antibody based selection procedure. Standard expression cloning methods are

applied including, e.g., FACS staining of membrane associated expression product. The antibodies are used to identify clones producing a recognized protein. Alternatively, antibodies are used to purify a 61164 or 331D5 chemokine chemokine, with protein sequencing and standard means to isolate a gene encoding that protein.

Genomic sequence based methods will also allow for identification of sequences naturally available, or otherwise, which exhibit homology to the provided sequences. Tissue distribution data may also be derived therefrom.

III. Isolation of a mouse 61164 or 331D5 chemokine clone

Similar methods are used as above to isolate an appropriate mouse chemokine gene. Similar source materials as indicated above are used to isolate natural genes, including genetic, polymorphic, allelic, or strain variants. Species variants are also isolated using similar methods.

IV. Isolation of another rodent or primate 61164 or 331D5 chemokine clone

An appropriate source is selected as above. Similar methods are utilized to isolate a species variant, though the level of similarity will typically be lower for an evolutionarily divergent chemokine as compared to a human to mouse sequence.

V. Expression; purification; characterization

With an appropriate clone from above, the coding sequence is inserted into an appropriate expression vector. This may be in a vector specifically selected for a prokaryote, yeast, insect, or higher vertebrate, e.g., mammalian expression system. Standard methods are applied to produce the gene product, preferably as a soluble secreted molecule, but will, in certain instances, also be made as an intracellular protein. Intracellular proteins typically require cell lysis to recover the protein, and insoluble inclusion bodies are a common starting material for further purificiation.

With a clone encoding a vertebrate 61164 or 331D5 chemokine, recombinant production means are used, although natural forms may be purified from appropriate sources. The protein product is purified by standard methods of protein purification, in certain cases, e.g., coupled with immunoaffinity methods. Immunoaffinity methods are used either as a purification step, as described above, or as a detection assay to determine the separation properties of the protein.

Preferably, the protein is secreted into the medium, and the soluble product is purified from the medium in a soluble form. Alternatively, as described above, inclusion bodies from prokaryotic expression systems are a useful source of material. Typically, the insoluble protein is solubilized from the inclusion bodies and refolded using standard methods. Purification methods are developed as described above.

The product of the purification method described above is characterized to determine many structural features. Standard physical methods are applied, e.g., amino acid analysis and protein sequencing. The resulting protein is subjected to CD spectroscopy and other spectroscopic methods, e.g., NMR, ESR, mass spectroscopy, etc. The product is characterized to determine its molecular form and size, e.g., using gel chromatography and similar techniques. Understanding of the chromatographic properties will lead to more gentle or efficient purification methods.

Prediction of glycosylation sites may be made, e.g., as reported in Hansen, et al. (1995) Biochem. J. 308:801-813. Signal cleavage sites may be predicted, and structure prediction programs exist, e.g., PHD (Rost and Sander (1994) Proteins 19:55-72) and DSC (King and Sternberg (1996) Protein Sci. 5:2298-2310).

VI. Preparation of antibodies against vertebrate 61164 or 331D5 chemokine

With protein produced, as above, animals are immunized to produce antibodies. Antigens may be synthetic peptides, peptide fragments, recombinant proteins, or naturally

purified proteins. Antigens may be native or denatured. Short peptides may be conjugated to a carrier, e.g. keyhole limpet and hemocyanin. Polyclonal antiserum is raised using non-purified antigen, though the resulting serum will exhibit higher background levels. Preferably, the antigen is purified using standard protein purification techniques, including, e.g., affinity chromatography using polyclonal serum indicated above. Presence of specific antibodies is detected using defined synthetic peptide fragments.

Polyclonal serum is raised against a purified antigen, purified as indicated above, or using, e.g., a plurality of, synthetic peptides. A series of overlapping synthetic peptides which encompass all of the full length sequence, if presented to an animal, will produce serum recognizing most linear epitopes on the protein. Such an antiserum is used to affinity purify protein, which is, in turn, used to introduce intact full length protein into another animal to produce another antiserum preparation.

Similar techniques are used to generate induce monoclonal antibodies to either unpurified antigen, or, preferably, purified antigen.

VII. Cellular and tissue distribution

Distribution of the protein or gene products are determined, e.g., using immunohistochemistry with an antibody reagent, as produced above, or by screening for nucleic acids encoding the chemokine. Either hybridization or PCR methods are used to detect DNA, cDNA, or message content. Histochemistry allows determination of the specific cell types within a tissue which express higher or lower levels of message or DNA. Antibody techniques are useful to quantitate protein in a biological sample, including a liquid or tissue sample. Immunoassays are developed to quantitate protein.

Preliminary expression data for human 61164 indicates its presence in fetal spleen and tonsilar tissues. Low expression can be detected in small intestine, indicating some specificity in lymphoid tissues. 61164 has not been detected in splenocytes.

Southern Analysis: DNA (5 µg) from a primary-amplified cDNA library was digested with appropriate restriction enzymes to release the inserts, run on a 1% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH).

Samples for primate mRNA isolation include: peripheral blood mononuclear cells (monocytes, T cells, NK cells, granulocytes, B cells), resting (T100); peripheral blood mononuclear cells, activated with anti-CD3 for 2, 6, 12 h pooled (T101); T cell, TH0 clone Mot 72, resting (T102); T cell, TH0 clone Mot 72, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T103); T cell, TH0 clone Mot 72, anergic treated with specific peptide for 2, 7, 12 h pooled (T104); T cell, TH1 clone HY06, resting (T107); T cell, TH1 clone HY06, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T108); T cell, TH1 clone HY06, anergic treated with specific peptide for 2, 6, 12 h pooled (T109); T cell, TH2 clone HY935, resting (T110); T cell, TH2 clone HY935, activated with anti-CD28 and anti-CD3 for 2, 7, 12 h pooled (T111); T cells CD4+CD45RO- T cells polarized 27 days in anti-CD28, IL-4, and anti IFN-γ, TH2 polarized, activated with anti-CD3 and anti-CD28 4 h (T116); ; T cell tumor lines Jurkat and Hut78, resting (T117); T cell clones, pooled AD130.2, Tc783.12, Tc783.13, Tc783.58, Tc782.69, resting (T118); T cell random γδ T cell clones, resting (T119); Splenocytes, resting (B100); Splenocytes, activated with anti-CD40 and IL-4 (B101); B cell EBV lines pooled WT49, RSB, JY, CVIR, 721.221, RM3, HSY, resting (B102); B cell line JY, activated with PMA and ionomycin for 1, 6 h pooled (B103); NK 20 clones pooled, resting (K100); NK 20 clones pooled, activated with PMA and ionomycin for 6 h (K101); NKL clone, derived from peripheral blood of LGL leukemia patient, IL-2 treated (K106); NK cytotoxic clone 640-A30-1, resting (K107); hematopoietic precursor line TF1, activated with PMA and ionomycin for 1, 6 h pooled (C100); U937 premonocytic line, resting (M100); U937 premonocytic line, activated with PMA and ionomycin for 1, 6 h pooled (M101); elutriated monocytes, activated with LPS, IFNγ, anti-IL-10 for 1, 2, 6, 12, 24 h pooled (M102); elutriated monocytes, activated with LPS, IFNγ,

IL-10 for 1, 2, 6, 12, 24 h pooled (M103); elutriated monocytes, activated with LPS, IFNγ, anti-IL-10 for 4, 16 h pooled (M106); elutriated monocytes, activated with LPS, IFNγ, IL-10 for 4, 16 h pooled (M107); elutriated monocytes, activated LPS for 1 h (M108); elutriated monocytes, activated LPS for 6 h (M109); DC 70% CD1a+, from CD34+ GM-CSF, TNFα 12 days, resting (D101); DC 70% CD1a+, from CD34+ GM-CSF, TNFα 12 days, activated with PMA and ionomycin for 1 hr (D102); DC 70% CD1a+, from CD34+ GM-CSF, TNFα 12 days, activated with PMA and ionomycin for 6 hr (D103); DC 95% CD1a+, from CD34+ GM-CSF, TNFα 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D104); DC 95% CD14+, ex CD34+ GM-CSF, TNFα 12 days FACS sorted, activated with PMA and ionomycin 1, 6 hr pooled (D105); DC CD1a+ CD86+, from CD34+ GM-CSF, TNFα 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D106); DC from monocytes GM-CSF, IL-4 5 days, resting (D107); DC from monocytes GM-CSF, IL-4 5 days, resting (D108); DC from monocytes GM-CSF, IL-4 5 days, activated LPS 4, 16 h pooled (D109); DC from monocytes GM-CSF, IL-4 5 days, activated TNFα, monocyte supe for 4, 16 h pooled (D110); leiomyoma L11 benign tumor (X101); normal myometrium M5 (O115); malignant leiomyosarcoma GS1 (X103); lung fibroblast sarcoma line MRC5, activated with PMA and ionomycin for 1, 6 h pooled (C101); kidney epithelial carcinoma cell line CHA, activated with PMA and ionomycin for 1, 6 h pooled (C102); kidney fetal 28 wk male (O100); lung fetal 28 wk male (O101); liver fetal 28 wk male (O102); heart fetal 28 wk male (O103); brain fetal 28 wk male (O104); gallbladder fetal 28 wk male (O106); small intestine fetal 28 wk male (O107); adipose tissue fetal 28 wk male (O108); ovary fetal 25 wk female (O109); uterus fetal 25 wk female (O110); testes fetal 28 wk male (O111); spleen fetal 28 wk male (O112); adult placenta 28 wk (O113); and tonsil inflamed, from 12 year old (X100).

Initial analysis of the human 331D5 showed it to be expressed in 6 h PMA/ionomycin activated 70% CD1a+ DC derived from CD34+ stem cells after 12 days culturing in GM-CSF and TNFα; resting DC derived from elutriated blood monocytes after 5 days culture in GM-CSF and IL-4; 4 and 16 h pooled

TNFα, IL-1α and monocyte supernatant activated DC derived from elutriated blood monocytes after 5 days culture in GM-CSF and IL-4; and resting 70% CD1a+ DC derived from CD34+ stem cells after 12 days culturing in GM-CSF and TNFα. Signal was detectable in resting HY06 TH1 T cell clone; 6 h LPS activated elutriated monocytes; anergic HY06 TH1 T cell clone treated with specific antigenic peptide for 2, 6, 12 h and pooled; 1 h PMA/ionomycin activated 70% CD1a+ DC derived from CD34+ stem cells after 12 days culturing in GM-CSF and TNFα; 2, 7, 12 h pooled activated with anti-CD28 and anti-CD3 HY935 TH2 T cell clone; and 1, 2, 6, 12, 24 h pooled activated LPS, IFNγ, and anti-IL-10 elutriated monocytes. Further analysis has shown the human 331D5 to be expressed in dendritic cell (DC) populations, e.g., 6 h PMA/ionomycin activated 70% CD1a+ DC derived from CD34+ stem cells after 12 days culturing in GM-CSF and TNFα; 4 and 16 h pooled TNFα, IL-1α and monocyte supernatant activated DC derived from elutriated blood monocytes after 5 days culture in GM-CSF and IL-4; 4 and 16 h pooled LPS activated DC from elutriated blood monocytes after 5 days culture in GM-CSF and IL-4; resting 70% CD1a+ derived from CD34+ stem cells after 12 days culturing in GM-CSF and TNFα; resting pooled WT49, RSB, JY, CIR, 721.221, RM3, and HSY EBV tumor B cell lines; and anti-CD40 and IL-4 activated total splenocytes. Barely detectable signals were observed with anergic HY06 treated with specific antigenic peptide for 2, 6, and 12 h pooled TH1 T cell line; resting TH1 HY06 T cell clone; 2, 6, 12 h pooled anti-CD3 and PMA activated PBMC cells; resting PBMC; 6 h LPS activated elutriated monocytes; 1 h PMA/ionomycin activated 70% CD1a+ DC derived from CD34+ stem cells after 12 days culturing in GM-CSF and TNFα; 2, 6, and 12 h pooled anti-CD28 and anti-CD3 activated HY06 TH1 T cell clone; and resting HY935 TH2 T cell clone.

Samples for rodent mRNA isolation include: resting mouse fibroblastic L cell line (C200); Braf:ER (Braf fusion to estrogen receptor) transfected cells, control (C201); T cells, TH1 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IFN-g and anti IL-4; T200); T cells, TH2 polarized (Mel14 bright, CD4+ cells from spleen,

polarized for 7 days with IL-4 and anti-IFN-γ; T201); T cells, highly TH1 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T202); T cells, highly TH2 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T203); CD44- CD25+ pre T cells, sorted from thymus (T204); TH1 T cell clone D1.1, resting for 3 weeks after last stimulation with antigen (T205); TH1 T cell clone D1.1, 10 µg/ml ConA stimulated 15 h (T206); TH2 T cell clone CDC35, resting for 3 weeks after last stimulation with antigen (T207); TH2 T cell clone CDC35, 10 µg/ml ConA stimulated 15 h (T208); Mel 14+ naive T cells from spleen, resting (T209); Mel14+ T cells, polarized to Th1 with IFN-g/IL-12/anti-IL-4 for 6, 12, 24 h pooled (T210); Mel 14+ T cells, polarized to Th2 with IL-4/anti-IFN-γ for 6, 13, 24 h pooled (T211); unstimulated mature B cell leukemia cell line A20 (B200); unstimulated B cell line CH12 (B201); unstimulated large B cells from spleen (B202); B cells from total spleen, LPS activated (B203); metrizamide enriched dendritic cells from spleen, resting (D200); dendritic cells from bone marrow, resting (D201); monocyte cell line RAW 264.7 activated with LPS 4 h (M200); bone-marrow macrophages derived with GM and M-CSF (M201); macrophage cell line J774, resting (M202); macrophage cell line J774 + LPS + anti-IL-10 at 0.5, 1, 3, 6, 12 h pooled (M203); macrophage cell line J774 + LPS + IL-10 at 0.5, 1, 3, 5, 12 h pooled(M204); aerosol challenged mouse lung tissue, Th2 primere, aerosol OVA challenge 7, 14, 23 h pooled (see Garlisi, et al. (1995) Clinical Immunology and Immunopathology 75:75-83; X206); Nippostrongulus-infected lung tissue (see Coffman, et al. (1989) Science 245:308-310; X200); total adult lung, normal (O200); total lung, rag-1 (see Schwarz, et al. (1993) Immunodeficiency 4:249-252; O205); IL-10 K.O. spleen (see Kuhn, et al. (1991) Cell 75:263-274; X201); total adult spleen, normal (O201); total spleen, rag-1 (O207); IL-10 K.O. Peyer's patches (O202); total Peyer's patches, normal (O210); IL-10 K.O. mesenteric lymph nodes (X203); total mesenteric lymph nodes, normal (O211); IL-10 K.O. colon (X203); total colon, normal (O212); NOD mouse pancreas (see Makino, et al.

(1980) Jikken Dobutsu 29:1-13; X205); total thymus, rag-1 (O208); total kidney, rag-1 (O209); total heart, rag-1 (O202); total brain, rag-1 (O203); total testes, rag-1 (O204); total liver, rag-1 (O206); rat normal joint tissue (O300); and rat arthritic joint tissue (X300).

Analysis of the mouse counterpart of the 331D5 has shown it to be expressed highly in the B cell line CH12; dendritic cells from spleen (resting) or bone marrow (resting); and TH2 polarized T cells. It is also easily detectable in the monocyte cell line RAW264.7; large B cells from spleen; and Mel 14+ T cells polarized with IL-4. A detectable signal appears in highly polarized TH1 cells; highly polarized TH2 cells; total normal mesenteric lymph nodes; and RAG-1 total testes.

VIII. Microchemotaxis assays

The pro-migratory activities of 61164 or 331D5 chemokine are assessed in microchemotaxis assays. See, e.g., Bacon, et al. (1988) Br. J. Pharmacol. 95:966-974.

IX. Biological activities, direct and indirect

A robust and sensitive assay is selected as described above, e.g., on immune cells, neuronal cells, or stem cells. Chemokine is added to the assay in increasing doses to see if a dose response is detected. For example, in a proliferation assay, cells are plated out in plates. Appropriate culture medium is provided, and chemokine is added to the cells in varying amounts. Growth is monitored over a period of time which will detect either a direct effect on the cells, or an indirect effect of the chemokine.

Alternatively, an activation assay or attraction assay is used. An appropriate cell type is selected, e.g, hematopoietic cells, myeloid (macrophages, neutrophils, polymorphonuclear cells, etc.) or lymphoid (T cell, B cell, or NK cells), neural cells (neurons, neuroglia, oligodendrocytes, astrocytes, etc.), or stem cells, e.g., progenitor cells which differentiate to other cell types, e.g., gut crypt cells and undifferentiated cell types.

Other assays will be those which have been demonstrated with other chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109. Calcium flux can be assayed in response to contacting by chemokine. The calcium flux response of various G-protein coupled receptors may be screened for response to chemokine contacting. See, e.g. Van Riper, et al., 1993 J. Exp. Med. 177:851-856.

X. Structure activity relationship

Information on the criticality of particular residues is determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many different variants at determined positions, e.g., at the positions identified above, and evaluating biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to determine the residues which can be substituted to either retain, block, or modulate biological activity.

Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among individuals, or across strains or species. Samples from selected individuals are analysed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

XI. Screening for agonists/antagonists

Agonists or antagonists are screened for ability to induce or block biological activity. The candidate compounds, e.g, sequence variants of natural 61164 or 331D5 chemokine, are assayed for their biological activities. Alternatively, compounds are screened, alone or in combinations, to determine effects on biological activity.

XII. Isolation of a Receptor for 61164 or 331D5 chemokine

A 61164 or 331D5 chemokine can be used as a specific binding reagent to identify its binding partner, by taking

advantage of its specificity of binding, much like an antibody would be used. A binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. The typical chemokine receptor is a seven transmembrane receptor.

The binding composition, e.g., chemokine, is used to screen an expression library made from a cell line which expresses a binding partner, i.e. receptor. Standard staining techniques are used to detect or sort intracellular or surface expressed receptor, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by various staining or immunofluorescence procedures. See also McMahan, et al. (1991) EMBO J. 10:2821-2832.

For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at 2-3 x 10^5 cells per chamber in 1.5 ml of growth media. Incubate overnight at 37° C.

On day 1 for each sample, prepare 0.5 ml of a solution of 66 µg/ml DEAE-dextran, 66 µM chloroquine, and 4 µg DNA in serum free DME. For each set, a positive control is prepared, e.g., of human 61164 or 331D5 chemokine cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37° C. Remove the medium and add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the cells are fixed and stained. Rinse the cells twice with Hank's Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may be stored at -80° C after all liquid is removed. For each chamber, 0.5 ml incubations are performed as follows. Add HBSS/saponin (0.1%) with 32 µl/ml of 1 M NaN_3 for 20 min. Cells are then washed with HBSS/saponin 1X. Add chemokine or chemokine/antibody complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. If appropriate, add first antibody for 30 min. Add second

antibody, e.g., Vector anti-mouse antibody, at 1/200 dilution, and incubate for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish peroxidase solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. Wash cells twice with HBSS, second wash for 2 min, which closes cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H_2O_2 per 5 ml of glass distilled water. Carefully remove chamber and rinse slide in water. Air dry for a few minutes, then add 1 drop of Crystal Mount and a cover slip. Bake for 5 min at 85-90° C.

Evaluate positive staining of pools and pregressively subclone to isolation of single genes responsible for the binding.

Alternatively, chemokine reagents are used to affinity purify or sort out cells expressing a receptor. See, e.g., Sambrook, et al. or Ausubel, et al.

Another strategy is to screen for a membrane bound receptor by panning. The receptor cDNA is constructed as described above. The ligand can be immobilized and used to immobilize expressing cells. Immobilization may be achieved by use of appropriate antibodies which recognize, e.g., a FLAG sequence of a chemokine fusion construct, or by use of antibodies raised against the first antibodies. Recursive cycles of selection and amplification lead to enrichment of appropriate clones and eventual isolation of receptor expressing clones.

Phage expression libraries can be screened by chemokine. Appropriate label techniques, e.g., anti-FLAG antibodies, will allow specific labeling of appropriate clones.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

SEQUENCE SUBMISSION

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: SCHERING CORPORATION
(B) STREET: 2000 GALLOPING HILL ROAD
(C) CITY: KENILWORTH
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07033
(G) Telephone: (908) 298-2987
(H) Telefax: (908) 298-5388

(ii) TITLE OF INVENTION: MAMMALIAN CHEMOKINES; RELATED REAGENTS

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: not yet assigned

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/025,724
(B) FILING DATE: 10-SEP-1996

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 301 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAAGTTCATC TCGACATCTC TGCTTCTCAT GCTGCTGGTC AGACGCTCTC TCCAGTCCAA 60

GGTGTTCTGG AGGTCTATTA CACAAGCTTG AGGTGTAGAT GTGTCCAAGA GAGCTCAGTC 120

TTTATCCCTA GACGCTTCAT TGATCGAATT CAAATCTTGC CCCGTGGGAA TGGTTGTCCA 180

AGAAAAGAAA TCATAGTCTG GAAGAAGAAC AAGTCAATTG TGTGTGTGGA CCCTCAAGCT 240

GAATGGATAC AAAGAATGAT GGAAGTATTG AGAAAAAGAA GTTCTTCAAC TCTACCAGTT 300

C 301

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 32..34
(D) OTHER INFORMATION: /note= "CXC chemokine motif"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val His Leu Asp Ile Ser Ala Ser His Ala Ala Gly Gln Thr Leu
1 5 10 15

Ser Pro Val Gln Gly Val Leu Glu Val Tyr Tyr Thr Ser Leu Arg Cys
20 25 30

Arg Cys Val Gln Glu Ser Ser Val Phe Ile Pro Arg Arg Phe Ile Asp
35 40 45

Arg Ile Gln Ile Leu Pro Arg Gly Asn Gly Cys Pro Arg Lys Glu Ile
50 55 60

Ile Val Trp Lys Lys Asn Lys Ser Ile Val Cys Val Asp Pro Gln Ala
65 70 75 80

Glu Trp Ile Gln Arg Met Met Glu Val Leu Arg Lys Arg Ser Ser Ser
85 90 95

Thr Leu Pro Val
100

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Lys Phe Ile Ser Thr Ser Leu Leu Leu Met Leu Leu Val Arg Arg Ser
1 5 10 15

Leu Gln Ser Lys Val Phe Trp Arg Ser Ile Thr Gln Ala Xaa Gly Val
20 25 30

Asp Val Ser Lys Arg Ala Gln Ser Leu Ser Leu Asp Ala Ser Leu Ile
35 40 45

Glu Phe Lys Ser Cys Pro Val Gly Met Val Val Gln Glu Lys Lys Ser
50 55 60

Xaa Ser Gly Arg Arg Thr Ser Gln Leu Cys Val Trp Thr Leu Lys Leu
65 70 75 80

Asn Gly Tyr Lys Glu Xaa Trp Lys Tyr Xaa Glu Lys Glu Val Leu Gln
85 90 95

Leu Tyr Gln Phe
100

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Ser Ser Arg His Leu Cys Phe Ser Cys Cys Trp Ser Asp Ala Leu
1 5 10 15

Ser Ser Pro Arg Cys Ser Gly Gly Leu Leu His Lys Leu Glu Val Xaa
20 25 30

Met Cys Pro Arg Glu Leu Ser Leu Tyr Pro Xaa Thr Leu His Xaa Ser
35 40 45

Asn Ser Asn Leu Ala Pro Trp Glu Trp Leu Ser Lys Lys Arg Asn His
50 55 60

Ser Leu Glu Glu Glu Gln Val Asn Cys Val Cys Gly Pro Ser Ser Xaa
65 70 75 80

Met Asp Thr Lys Asn Asp Gly Ser Ile Glu Lys Lys Lys Phe Phe Asn
85 90 95

Ser Thr Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1136 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..327

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 67..327

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG AAG TTC ATC TCG ACA TCT CTG CTT CTC ATG CTG CTG GTC AGC AGC          48
Met Lys Phe Ile Ser Thr Ser Leu Leu Leu Met Leu Leu Val Ser Ser
-22  -20                 -15                 -10

CTC TCT CCA GTC CAA GGT GTT CTG GAG GTC TAT TAC ACA AGC TTG AGG          96
Leu Ser Pro Val Gln Gly Val Leu Glu Val Tyr Tyr Thr Ser Leu Arg
 -5                   1                   5                  10

TGT AGA TGT GTC CAA GAG AGC TCA GTC TTT ATC CCT AGA CGC TTC ATT         144
Cys Arg Cys Val Gln Glu Ser Ser Val Phe Ile Pro Arg Arg Phe Ile
                15                  20                  25

GAT CGA ATT CAA ATC TTG CCC CGT GGG AAT GGT TGT CCA AGA AAA GAA         192
Asp Arg Ile Gln Ile Leu Pro Arg Gly Asn Gly Cys Pro Arg Lys Glu
            30                  35                  40

ATC ATA GTC TGG AAG AAG AAC AAG TCA ATT GTG TGT GTG GAC CCT CAA         240
Ile Ile Val Trp Lys Lys Asn Lys Ser Ile Val Cys Val Asp Pro Gln
        45                  50                  55

GCT GAA TGG ATA CAA AGA ATG ATG GAA GTA TTG AGA AAA AGA AGT TCT         288
Ala Glu Trp Ile Gln Arg Met Met Glu Val Leu Arg Lys Arg Ser Ser
    60                  65                  70

TCA ACT CTA CCA GTT CCA GTG TTT AAG AGA AAG ATT CCC TGATGCTGAT          337
Ser Thr Leu Pro Val Pro Val Phe Lys Arg Lys Ile Pro
 75                  80                  85

ATTTCCACTA AGAACACCTG CATTCTTCCC TTATCCCTGC TCTGGATTTT AGTTTTGTGC       397

TTAGTTAAAT CTTTTCCAGG AAAAAGAACT TCCCCATACA AATAAGCATG AGACTATGTA       457

AAAATAACCT TGCAGAAGCT GGATGGGGCA AACTCAAGCT TCTTCACTCA CAGCACCCTA       517

TATACACTTG GAGTTTGCAT TCTTATTCAT CAGGGAGGAA AGTTTCTTTG AAAATAGTTA       577

TTCAGTTATA AGTAATACAG GATTATTTTG ATTATATACT TGTTGTTTAA TGTTTAAAAT       637

TTCTTAGAAA ACAATGGAAT GAGAATTTAA GCTCAAATTT GAACATGTGG CTTGAATTAA       697

GAAGAAAATT ATGGCATATA TTAAAAGCAG GCTTCTATGA AAGACTCAAA AAGCTGCCTG       757

GGAGGCAGAT GGAACTTGAG CCTGTCAAGA GGCAAAGGAA TCCATGTAGT AGATATCCTC       817

TGCTTAAAAA CTCACTACGG AGGAGAATTA AGTCCTACTT TTAAAGAATT TCTTTATAAA       877

ATTTACTGTC TAAGATTAAT AGCATTCGAA GATCCCCAGA CTTCATAGAA TACTCAGGGA       937

AAGCATTTAA AGGGTGATGT ACACATGTAT CCTTTCACAC ATTTGCCTTG ACAAACTTCT       997

TTCACTCACA TCTTTTTCAC TGACTTTTTT TGTGGGGGGC GGGGCCGGGG GGACTCTGGT      1057

ATCTAATTCT TTAATGATTC CTATAAATCT AATGACATTC AATAAAGTTG AGCAAACATT      1117

TTAAAAAAAA AAAAAAAAA                                                  1136
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Lys Phe Ile Ser Thr Ser Leu Leu Leu Met Leu Leu Val Ser Ser
-22      -20                 -15                 -10

Leu Ser Pro Val Gln Gly Val Leu Glu Val Tyr Tyr Thr Ser Leu Arg
     -5                  1                   5                  10

Cys Arg Cys Val Gln Glu Ser Ser Val Phe Ile Pro Arg Arg Phe Ile
                 15                  20                  25

Asp Arg Ile Gln Ile Leu Pro Arg Gly Asn Gly Cys Pro Arg Lys Glu
             30                  35                  40

Ile Ile Val Trp Lys Lys Asn Lys Ser Ile Val Cys Val Asp Pro Gln
         45                  50                  55

Ala Glu Trp Ile Gln Arg Met Met Glu Val Leu Arg Lys Arg Ser Ser
     60                  65                  70

Ser Thr Leu Pro Val Pro Val Phe Lys Arg Lys Ile Pro
 75                  80                  85
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GGTTGAACTC CACCTCCAGG CAGA ATG AGG CTC AGC ACA GCA ACG CTG CTT          51
                           Met Arg Leu Ser Thr Ala Thr Leu Leu
                           -21 -20                 -15

CTC CTC CTG GCC AGC TGC CTC TCT CCA GGC CAC GGT ATT CTG GAA GCC          99
Leu Leu Leu Ala Ser Cys Leu Ser Pro Gly His Gly Ile Leu Glu Ala
        -10                 -5                  1

CAT TAC ACA AAC TTA AAA TGT AGG TGT TCT GGA GTG ATT TCA ACT GTT         147
His Tyr Thr Asn Leu Lys Cys Arg Cys Ser Gly Val Ile Ser Thr Val
  5                 10                  15                  20

GTC GGT CTA AAC ATC ATA GAT CGG ATT CAA GTT ACG CCC CCT GGG AAT         195
Val Gly Leu Asn Ile Ile Asp Arg Ile Gln Val Thr Pro Pro Gly Asn
                  25                  30                  35

GGC TGC CCC AAA ACT GAA GTT GTG ATC TGG ACC AAG ATG AAG AAA GTT         243
Gly Cys Pro Lys Thr Glu Val Val Ile Trp Thr Lys Met Lys Lys Val
              40                  45                  50

ATA TGT GTG AAT CCT CGT GCC AAA TGG TTA CAA AGA TTA TTA AGA CAT         291
Ile Cys Val Asn Pro Arg Ala Lys Trp Leu Gln Arg Leu Leu Arg His
          55                  60                  65

GTC CAA AGC AAA AGT CTG TCT TCA ACT CCC CAA GCT CCA GTG AGT AAG         339
Val Gln Ser Lys Ser Leu Ser Ser Thr Pro Gln Ala Pro Val Ser Lys
      70                  75                  80

AGA AGA GCT GCC TGAAGCCACT ATCATCTCAA AAGACACACC TGCACCTTTT             391
Arg Arg Ala Ala
 85
```

TTTTTATCCC TGCTCTGAAT TTTAGATATG TTCTTAGTTA AAGAATTTCC AAGAAAATAA 451

CTCCCCTCTA CAAACAAACC AACTAAAAAA AAAAACCCA 490

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Arg Leu Ser Thr Ala Thr Leu Leu Leu Leu Ala Ser Cys Leu
-21 -20                 -15                 -10

Ser Pro Gly His Gly Ile Leu Glu Ala His Tyr Thr Asn Leu Lys Cys
 -5                   1                   5                  10

Arg Cys Ser Gly Val Ile Ser Thr Val Val Gly Leu Asn Ile Ile Asp
             15                  20                  25

Arg Ile Gln Val Thr Pro Pro Gly Asn Gly Cys Pro Lys Thr Glu Val
         30                  35                  40

Val Ile Trp Thr Lys Met Lys Lys Val Ile Cys Val Asn Pro Arg Ala
     45                  50                  55

Lys Trp Leu Gln Arg Leu Leu Arg His Val Gln Ser Lys Ser Leu Ser
 60                  65                  70                  75

Ser Thr Pro Gln Ala Pro Val Ser Lys Arg Arg Ala Ala
                 80                  85
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 614 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..258

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 46..258

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CTC CTG GTT GTC CTC GTC CTC CTT GCT GTG GCG CTT CAA GCA ACT GAG      48
Leu Leu Val Val Leu Val Leu Leu Ala Val Ala Leu Gln Ala Thr Glu
-15                 -10                 -5                  1
```

```
GCA GGC CCC TAC GGC GCC AAC ATG GAA GAC AGC GTC TGC TGC CGT GAT          96
Ala Gly Pro Tyr Gly Ala Asn Met Glu Asp Ser Val Cys Cys Arg Asp
                5                  10                  15

TAC GTC CGT TAC CGT CTG CCC CTG CGC GTG GTG AAA CAC TTC TAC TGG         144
Tyr Val Arg Tyr Arg Leu Pro Leu Arg Val Val Lys His Phe Tyr Trp
            20                  25                  30

ACC TCA GAC TCC TGC CCG AGG CCT GGC GTG GTG TTG CTA ACC TTC AGG         192
Thr Ser Asp Ser Cys Pro Arg Pro Gly Val Val Leu Leu Thr Phe Arg
        35                  40                  45

GAT AAG GAG ATC TGT GCC GAT CCC AGA GTG CCC TGG GTG AAG ATG ATT         240
Asp Lys Glu Ile Cys Ala Asp Pro Arg Val Pro Trp Val Lys Met Ile
    50                  55                  60                  65

CTC AAT AAG CTG AGC CAA TGAAGAGCCT ACTCTGATGA CCGTGGCCTT               288
Leu Asn Lys Leu Ser Gln
                70

GGCTCCTCCA GGAAGCTCAG GAGCCCTACC TCCCTGCCAT TATTGCTGCT CCCCGCCAGA      348

AGCCTGTGCC AACTCTCTGC ATTCCCTGAT CTCCATCCCT GTGGCTGTCA CCCTTGGTCA      408

CCTCCGTGCT GTCACTGCCA TCTCCCCCCT GACCCTCTAA CCCATCCTCT GCTCCCTCCC      468

TGCAGTCAGA AGGTCCTGTT CCCATCAGCG ATCCCTGCTT AAACCTCCCA TGAATCCCCA      528

CTGCCTAAGC TGAAGTCAGT CTCCCAAGCC TGGCATGTTG GCCTCTGGAT TTGGGTCCAT      588

CTCTGTTCCA GCTGGCCACT TCCTTC                                           614
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Leu Leu Val Val Leu Val Leu Leu Ala Val Ala Leu Gln Ala Thr Glu
-15                 -10                 -5                  1

Ala Gly Pro Tyr Gly Ala Asn Met Glu Asp Ser Val Cys Cys Arg Asp
                5                  10                  15

Tyr Val Arg Tyr Arg Leu Pro Leu Arg Val Val Lys His Phe Tyr Trp
            20                  25                  30

Thr Ser Asp Ser Cys Pro Arg Pro Gly Val Val Leu Leu Thr Phe Arg
        35                  40                  45

Asp Lys Glu Ile Cys Ala Asp Pro Arg Val Pro Trp Val Lys Met Ile
    50                  55                  60                  65

Leu Asn Lys Leu Ser Gln
                70
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2930 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "residue 1560 may be A or G"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 48..326

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 120..326

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TGAATTCAGC GGCCGCTGAA TTCTAGCTGA GACATACAGG ACAGAGC ATG GCT CGC          56
                                                    Met Ala Arg
                                                    -24

CTA CAG ACT GCA CTC CTG GTT GTC CTC GTC CTC CTT GCT GTG GCG CTT          104
Leu Gln Thr Ala Leu Leu Val Val Leu Val Leu Leu Ala Val Ala Leu
-20                 -15                 -10

CAA GCA ACT GAG GCA GGC CCC TAC GGC GCC AAC ATG GAA GAC AGC GTC          152
Gln Ala Thr Glu Ala Gly Pro Tyr Gly Ala Asn Met Glu Asp Ser Val
 -5                  1                   5                  10

TGC TGC CGT GAT TAC GTC CGT TAC CGT CTG CCC CTG CGC GTG GTG AAA          200
Cys Cys Arg Asp Tyr Val Arg Tyr Arg Leu Pro Leu Arg Val Val Lys
             15                  20                  25

CAC TTC TAC TGG ACC TCA GAC TCC TGC CCG AGG CCT GGC GTG GTG TTG          248
His Phe Tyr Trp Thr Ser Asp Ser Cys Pro Arg Pro Gly Val Val Leu
         30                  35                  40

CTA ACC TTC AGG GAT AAG GAG ATC TGT GCC GAT CCC AGA GTG CCC TGG          296
Leu Thr Phe Arg Asp Lys Glu Ile Cys Ala Asp Pro Arg Val Pro Trp
     45                  50                  55

GTG AAG ATG ATT CTC AAT AAG CTG AGC CAA TGAAGAGCCT ACTCTGATGA           346
Val Lys Met Ile Leu Asn Lys Leu Ser Gln
 60                  65

CCGTGGCCTT GGCTCCTCCA GGAAGGCTCA GGAGCCCTAC CTCCCTGCCA TTATAGCTGC      406

TCCCCGCCAG AAGCCTGTGC CAACTCTCTG CATTCCCTGA TCTCCATCCC TGTGGCTGTC      466

ACCCTTGGTC ACCTCCGTGC TGTCACTGCC ATCTCCCCCC TGACCCCTCT AACCCATCCT      526

CTGCCTCCCT CCCTGCAGTC AGAGGGTCCT GTTCCCATCA GCGATTCCCC TGCTTAAACC      586

CTTCCATGAC TCCCCACTGC CCTAAGCTGA GGTCAGTCTC CCAAGCCTGG CATGTGGCCC      646
```

TCTGGATCTG GGTTCCATCT CTGTCTCCAG CCTGCCCACT TCCCTTCATG AATGTTGGGT 706

TCTAGCTCCC TGTTCTCCAA ACCCATACTA CACATCCCAC TTCTGGGTCT TTGCCTGGGA 766

TGTTGCTGAC ACTCAGAAAG TCCCACCACC TGCACATGTG TAGCCCCACC AGCCCTCCAA 826

GGCATTGCTC GCCCAAGCAG CTGGTAATTC CATTTCATGT ATTAGATGTC CCCTGGCCCT 886

CTGTCCCCTC TTAATAACCC TAGTCACAGT CTCCGCAGAT TCTTGGGATT TGGGGGTTTT 946

CTCCCCCACC TCTCCACTAG TTGGACCAAG GTTTCTAGCT AAGTTACTCT AGTCTCCAAG 1006

CCTCTAGCAT AGAGCACTGC AGACAGGCCC TGGCTCAGAA TCAGAGCCCA GAAAGTGGCT 1066

GCAGACAAAA TCAATAAAAC TAATGTCCCT CCCCTCTCCC TGCCAAAAGG CAGTTACATA 1126

TCAATACAGA GACTCAAGGT CACTAGAAAT GGGCCAGCTG GGTCAATGTG AAGCCCCAAA 1186

TTTGCCCAGA TTCACCTTTC TTCCCCCACT CCCTTTTTTT TTTTTTTTGA GATGGAGTTT 1246

CGCTCTTGTC ACCCACGCTG GAGTGCAATG GTGTGGTCTT GGCTTATTGA AGCCTCTGCC 1306

TCCTGGGTTC AAGTGATTCT CTTGCCTCAG CCTCCTGAGT AGCTGGGATT ACAGGTTCCT 1366

GCTACCACGC CCAGCTAATT TTTGTATTTT TAGTAGAGAC GAGGCTTCAC CATGTTGGCC 1426

AGGCTGGTCT CGAACTCCTG CCCTCAGGTA ATCCGCCCAC CTCAGCCTCC CAAAGTGCTG 1486

GGATTACAGG CGTGAGCCAC AGTGCCTGGC CTCTTCCCTC TCCCCACTGC CCCCCCCAAC 1546

TTTTTTTTTT TTTTATGGCA GGGTCTCACT CTGTCGCCCA GGCTGGAGTG CAGTGGCGTG 1606

ATCTCGGCTC ACTACAACCT CGACCTCCTG GGTTCAAGTG ATTCTCCCAC CCCAGCCTCC 1666

CAAGTAGCTG GGATTACAGG TGTGTGCCAC TACGGCTGGC TAATTTTTGT ATTTTTAGTA 1726

GAGACAGGTT TCACCATATT GGCCAGGCTG GTCTTGAACT CCTGACCTCA AGTGATCCAC 1786

CTTCCTTGTG CTCCCAAAGT GCTGAGATTA CAGGCGTGAG CTATCACACC CAGCCTCCCC 1846

CTTTTTTTCC TAATAGGAGA CTCCTGTACC TTTCTTCGTT TTACCTATGT GTCGTGTCTG 1906

CTTACATTTC CTTCTCCCCT CAGGCTTTTT TTGGGTGGTC CTCCAACCTC CAATACCCAG 1966

GCCTGGCCTC TTCAGAGTAC CCCCCATTCC ACTTTCCCTG CCTCCTTCCT TAAATAGCTG 2026

ACAATCAAAT TCATGCTATG GTGTGAAAGA CTACCTTTGA CTTGGTATTA TAAGCTGGAG 2086

TTATATATGT ATTTGAAAAC AGAGTAAATA CTTAAGAGGC CAAATAGATG AATGGAAGAA 2146

TTTTAGGAAC TGTGAGAGGG GGACAAGGTG AAGCTTTCCT GGCCCTGGGA GGAAGCTGGC 2206

TGTGGTAGCG TAGCGCTCTC TCTCTCTGTC TGTGGCAGGA GGCAAAGAGT AGGGTGTAAT 2266

TGAGTGAAGG AATCCTGGGT AGAGACCATT CTCAGGTGGT TGGGCCAGGC TAAAGACTGG 2326

GATTTGGGTC TATCTATGCC TTTCTGGCTG ATTTTTGTAG AGACGGGGTT TTGCCATGTT 2386

ACCCAGGCTG GTCTCAAACT CCTGGGCTCA AGCGATCCTC CTGGCTCAGC CTCCCAAAGT 2446

GCTGGGATTA CAGGCGTGAA TCACTGCGCC TGGCTTCCTC TTCCTCTTGA GAAATATTCT 2506

TTTCATACAG CAAGTATGGG ACAGCAGTGT CCCAGGTAAA GGACATAAAT GTTACAAGTG 2566

TCTGGTCCTT TCTGAGGGAG GCTGGTGCCG CTCTGCAGGG TATTTGAACC TGTGGAATTG 2626

GAGGAGGCCA TTTCACTCCC TGAACCCAGC CTGACAAATC ACAGTGAGAA TGTTCACCTT 2686

ATAGGCTTGC TGTGGGGCTC AGGTTGAAAG TGTGGGGAGT GACACTGCCT AGGCATCCAG 2746

CTCAGTGTCA TCCAGGGCCT GTGTCCCTCC CGAACCCAGG GTCAACCTGC CTGCCACAGG 2806

CACTAGAAGG ACGAATGTGC CTACTGCCCA TGAACGGGGC CCTCAAGCGT CCTGGGATCT 2866

CCTTCTCCCT CCTGTCCTGT CCTTGCCCCT CAGGACTGCT GGAAAATAAA TCCTTTAAAA 2926

TAGT 2930

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ala Arg Leu Gln Thr Ala Leu Leu Val Val Leu Val Leu Leu Ala
-24 -20 -15 -10

Val Ala Leu Gln Ala Thr Glu Ala Gly Pro Tyr Gly Ala Asn Met Glu
-5 1 5

Asp Ser Val Cys Cys Arg Asp Tyr Val Arg Tyr Arg Leu Pro Leu Arg
10 15 20

Val Val Lys His Phe Tyr Trp Thr Ser Asp Ser Cys Pro Arg Pro Gly
25 30 35 40

Val Val Leu Leu Thr Phe Arg Asp Lys Glu Ile Cys Ala Asp Pro Arg
45 50 55

Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu Ser Gln
60 65

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1944 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 165..440

(ix) FEATURE:
(A) NAME/KEY: mat_peptide

(B) LOCATION: 237..440

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
CCCCAGGGTT TAGAATTTAT GCTTTCCGGG TTCGTATGTT GTGTGGAATT GTGAGCGGAT      60

AACAATTTCA ACAACAGGAA ACAGCTATGA CATGATTACG AATTTAATAC GACTCACTAT     120

AGGGAATTTG GCCGTCGAGG CCAAGAATTC GGCACGAGGA CATC ATG GCT ACC CTG      176
                                                 Met Ala Thr Leu
                                                 -24

CGT GTC CCA CTC CTG GTG GCT CTC GTC CTT CTT GCT GTG GCA ATT CAG       224
Arg Val Pro Leu Leu Val Ala Leu Val Leu Leu Ala Val Ala Ile Gln
-20                 -15                 -10                  -5

ACC TCT GAT GCA GGT CCC TAT GGT GCC AAT GTG GAA GAC AGT ATC TGC       272
Thr Ser Asp Ala Gly Pro Tyr Gly Ala Asn Val Glu Asp Ser Ile Cys
                  1               5                  10

TGC CAG GAC TAC ATC CGT CAC CCT CTG CCA TCA CGT TTA GTG AAG GAG       320
Cys Gln Asp Tyr Ile Arg His Pro Leu Pro Ser Arg Leu Val Lys Glu
          15                  20                  25

TTC TTC TGG ACC TCA AAA TCC TGC CGC AAG CCT GGC GTT GTT TTG ATA       368
Phe Phe Trp Thr Ser Lys Ser Cys Arg Lys Pro Gly Val Val Leu Ile
     30                  35                  40

ACC GTC AAG AAC CGA GAT ATC TGT GCC GAT CCC AGG CAG GTC TGG GTG       416
Thr Val Lys Asn Arg Asp Ile Cys Ala Asp Pro Arg Gln Val Trp Val
 45                  50                  55                  60

AAG AAG CTA CTC CAT AAA CTG TCC TAGGGAGGAG GACCTGATGA CCATGGGTCT      470
Lys Lys Leu Leu His Lys Leu Ser
                 65

GGTGTGGTCC AGGGAGGCTC AGCAAGCCCT ATTCTTCTGC CATTCCAGCA AGAGCCTTGC     530

CAACGACGCC ACCTTTACTC ACCTCCATCC CCTGGGCTGT CACTCTGTGA GGCTCTGGTC     590

CCTCTACCTC CCCTCTATCC CTTCCAGCTT ATCCCCCTTC AATGTGGCAG CTGGGAAACA     650

CATTCAGGCC AGCCTTACCC AATGCCTACT CCCCACTGCT TTAGATGAGA CCAGCGTCCT     710

TGTTTTGATG CCCTGATCCT ATGATGCCTT CCCCATCCCC AGCCTTGGCC CCCTTCTCTT     770

CTTGCATGTA GGGAAGGCCC ATAGGTTTCA AATATGTGCT ACCTAGTTCC CTTTCTGGGG     830

GGTTCTAATA CCCAGCATGT TTTTCCTGCT GCAGGCACCT ATCCAGTGCC ACACACCTCC     890

CAAGTTTCTA TCAGTCCCAG TGGGCATCCA CCAAGCCCCA AACTTCAGAC TTCCTTGGCC     950

TCCACCTACT CTCAGTAGAA TTCTGGGAGT TTCAGGCTGG TCCACCAGGC CCCCCAGGGT    1010

TAGGCCAAGG TCCCCACCAG AGCTCCTCCT GTTTCTTGGT CTGCAGCACG GGGCAGGGAG    1070

CAAGGAGCAG GCTCAGAATC AGATTTCTTA AAGGAGCTGC AGACTCCATC AGTAAAAGGA    1130

ATCTTTCTCC CATCCCTGAA TATAAGGCAG TTTTCTGTCA ACACAGAGAC TCAGGTTGTT    1190

AGAAATGGCC ACATAGATCA ACTGTGAAAC CCTAAATTTA CCAAGAATCA ACTTCCACCC    1250
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CTCTTCAACC ACATGCTAGG GTCTTTTACT TTCTCTGCCC CACACCTTTG ACTCCTTGCC 1310

TGTGTAGCTG ATAGTCGAAG TTATGCTATG GTGTCAGTGA CTGCCACAGT TTGTTTGGTA 1370

TTATAAGCTA TAGTTATATT TATATAGGAA AGAGGATAAA TATATGTGAG GCCAAATAGA 1430

CGAACTGGAG AGTTTTAGGA TCTGGGGGCA GGAAGGGCCA TACAAAGTGA TACCTCAGAA 1490

AATAGATGGT TGTGGGAGCT GCTGCCAGTG GCAGAGTTAA CTTAAAGAAC TTAATTGAAA 1550

TTATTCTTGA GTGGCTGAGG CCAAGACAAG AATATAGAAC CCATTCTTGC TTCCCTGGAG 1610

ACAACAGTGG TCCCAGGGGA AGGAATAAAC CTTCTTGCTC CTCTGGAGGG AGCATGGCCT 1670

GGCTTAGCCG AGTGACTGGA CTGTGTGAGA TTGGGGGCAT CGCTTTTCCT CTCTGAGCCT 1730

CAGCTGACAG CATATGGGAC CACAAAGGGC TTGATCCAAA CCACAGGGAT TGACAGTGCC 1790

AGCCACAGCT GTGTCCAGGG CTCGTGTTCT GCCAGAAGGA GCACCTGGAC GACCAGGGCC 1850

ACCACTAGTG CTACTTTGCT CACTGCCCAT GCATGTCCTG AAGGTCCCTC CCCCTCCTCT 1910

CCTACTTCTG GGAAAATAAA TGCTCGCCAA TAAT 1944

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Ala Thr Leu Arg Val Pro Leu Leu Val Ala Leu Val Leu Leu Ala
-24             -20                 -15                 -10

Val Ala Ile Gln Thr Ser Asp Ala Gly Pro Tyr Gly Ala Asn Val Glu
            -5                  1                   5

Asp Ser Ile Cys Cys Gln Asp Tyr Ile Arg His Pro Leu Pro Ser Arg
     10                  15                  20

Leu Val Lys Glu Phe Phe Trp Thr Ser Lys Ser Cys Arg Lys Pro Gly
 25                  30                  35                  40

Val Val Leu Ile Thr Val Lys Asn Arg Asp Ile Cys Ala Asp Pro Arg
                45                  50                  55

Gln Val Trp Val Lys Lys Leu Leu His Lys Leu Ser
            60                  65
```

WHAT IS CLAIMED IS:

1. A substantially pure or recombinant polypeptide which:
(a) comprises a plurality of epitopes found on; and
(b) exhibits at least 90% sequence identity over a length of at least 12 contiguous amino acids to;
a polypeptide selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12 and SEQ ID NO: 14.

2. The polypeptide of Claim 1, wherein the polypeptide binds with specificity to an antibody generated against an immunogen selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 14.

3. The polypeptide of either Claim 1 or Claim 2, selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 14.

4. An isolated or recombinant nucleic acid which encodes a polypeptide of any of Claims 1-3.

5. The nucleic acid of Claim 4, selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 12.

6. A nucleic acid which:
a) hybridizes under wash conditions of 30° C and less than 2M salt to SEQ ID NO: 6 or 12; or
b) exhibits at least about 85% identity over a stretch of at least about 30 nucleotides to SEQ ID NO: 6 or SEQ ID NO: 12.

7. A vector comprising a nucleic acid of any of claims 4-6.

8. A host cell comprising a nucleic acid or vector of any of claims 4-7.

9. A method for making a polypeptide comprising culturing a host cell of claim 8 under conditions in which the nucleic acid or vector is expressed.

10. A binding compound comprising an antibody or antigen binding fragment therefrom which binds with specificity to a polypeptide of any of Claims 1-3.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/19, C07K 14/52, 16/24, C12N 15/11 //
C12N 15/62, A61K 38/19, C12Q 1/68

A3

(11) International Publication Number: **WO 98/11226**

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/US97/15315

(22) International Filing Date: 9 September 1997 (09.09.97)

(30) Priority Data:
60/025,724 10 September 1996 (10.09.96) US

(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

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(74) Agents: FOULKE, Cynthia, L. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, ID, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
7 May 1998 (07.05.98)

(54) Title: MAMMALIAN CHEMOKINES, RELATED REAGENTS

(57) Abstract

Novel CC and CXC chemokines from humans, reagents related thereto including purified proteins, specific antibodies and nucleic acids encoding these chemokines are provided. Also provided are methods of making and using said reagents and diagnostic kits.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/15315

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/52 C07K16/24 C12N15/11 //C12N15/62, A61K38/19,C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 17868 A (INCYTE PHARMA INC ;GUEGLER KARL J (US); HAWKINS PHILLIP R (US); WI) 13 June 1996 see SEQ ID 1 and 2; see page 4, line 10 - line 19; claims 1-8,12,13; figure 1 ---	1-10
X	WO 96 24668 A (HUMAN GENOME SCIENCES INC ;LI HAODONG (US)) 15 August 1996 see SEQ ID 3 and 4; see page 1, paragraph 1; claims 1,2,8-12,14,15,17-19; figure 2 see page 18, paragraph 4 see page 21, paragraph 4 - page 23, paragraph 4 --- -/--	1-4,6-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 February 1998	1 1 -03- 1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 97/15315

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WILKINSON A: "Human DNA sequence from cosmid U201H11, between markers DXS366 and DXS87 on chromosome X." EMBL SEQUENCE DATABASE, 26 February 1996, HEIDELBERG, GERMANY, XP002056622 from nucleotide 21.830 to 22.130	6-9
P,X	WO 96 39522 A (HUMAN GENOME SCIENCES INC ;LI HAODONG (US)) 12 December 1996 see SEQ ID 3 and 4; see claims 1,2,4,6-13; figure 2	1-10
P,X	WO 96 40923 A (ICOS CORP) 19 December 1996 see abstract; figure 1; examples 1,2,6,7,10,18 see the claims	1-10
P,X	WO 96 39521 A (HUMAN GENOME SCIENCES INC ;SMITHKLINE BEECHAM CORP (US); LI HAODON) 12 December 1996 see abstract; figure 1; examples 1-3 see the claims	1-10
P,X	MARRA M. ET AL.: "mr06f10.r1 Soares mouse 3NbMS Mus musculus cDNA clone 596683" EMBL SEQUENCE DATABASE, 13 December 1996, HEIDELBERG, GERMANY, XP002048796 see the whole document	4,7-9
P,X	MARRA M. ET AL.: "mr14f01.r1 Soares mouse 3NbMS Mus musculus cDNA clone 597433" EMBL SEQUENCE DATABASE, 15 January 1997, HEIDELBERG, GERMANY, XP002048797 see the whole document	4,7-9
A	SCHALL T J ET AL: "CHEMOKINES, LEUKOCYTE TRAFFICKING, AND INFLAMMATION" CURRENT OPINION IN IMMUNOLOGY, vol. 6, no. 6, December 1994, pages 865-873, XP000647638 see page 865, paragraph 3; figures 1-3 see page 868, paragraph 1 - page 869, paragraph 1	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/15315

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-10 (partially)

Chemokine 61164, nucleic acids derived therefrom, vectors and hosts containing them, and compounds binding to the chemokine.

2. Claims: 1-10 (partially)

Chemokine 311D5 polypeptides, nucleic acids derived therefrom, vectors and hosts containing them, and compounds binding to the chemokine.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Intern... ...pplication No
PCT/US 97/15315

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9617868 A	13-06-96	US 5633149 A	27-05-97
		AU 4597996 A	26-06-96
		CA 2207262 A	13-06-96
		EP 0797591 A	01-10-97
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		EP 0811059 A	10-12-97
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